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Literature Review of Pathobiology of Canine Hemangiosarcoma

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Declaration

I, Khalfa Ouail, hereby declare that this thesis is composed of my original work and contains no previously published work except where due reference was made in text. No part of this thesis has been submitted for a degree or other qualification at any other institution.

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List of Abbreviations

AGP	Alpha-1 acid glycoprotein
ANGPT	Angiopoietin
ARPC1A	Actin related protein 2/3 complex subunit 1A
BAK1	BCL2 Antagonist/Killer 1
Bcl-2	B-cell lymphoma 2
bFGF	Basic fibroblast growth factor
CAF	Carcinoma-associated fibroblast
CDKN2A/B	Cyclin-dependent kinase inhibitor 2A
CTL	Cytotoxic T lymphocytes
CXCR	Chemokine receptor
DAG	Diacylglycerol
DIC	Disseminated intravascular coagulation
ECM	Extracellular matrix
EPC	Endothelial progenitor cell
ET-1	Endothelin-1
FDP	Fibrinogen-degradation product
FGF	Fibroblastic growth factor
Flk-1	Fetal liver kinase 1
FNA	Fine needle aspirates
HSA	Hemangiosarcoma
IHC	Immunohistochemistry
IP3	Inositol triphosphate
KDR	Kinase insert domain receptor
K-ras	Kirsten rat sarcoma viral oncogene homolog
L-MTP-PE	Liposomal muramyl tripeptide phosphatidyl ethanolamine
LYVE-1	Lymphatic vessel endothelial receptor 1
MCT	Mast cell tumor
MDM2	Mouse double minute 2 homolog
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
MP	Microparticle

Myc	Myelocytomatosis oncogene
NOR	Nucleolar organizing regions
N-ras	Neuroblastoma rat sarcoma viral oncogene homolog
ORC1	Origin recognition complex 1
PDGF	Platelet-derived growth factor
PECAM-1	Platelet endothelial cell adhesion molecule-1
PIK	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol biphosphate
PLCG1	Phospholipase c, gamma 1
PlGF	Placenta growth factor
PROX-1	Prospero homeobox protein 1
PT	Prothrombin
PTEN	Phosphatase and tensin homolog
PTT	Partial thromboplastin time
PTS	Paraneoplastic syndrome
Ras	Rat sarcoma
RASA1	Ras p21 protein activator 1
RTK	Receptor tyrosine kinase
SDF-1	Stromal cell-derived factor-1
S1P1	Sphingosine-1-phosphate receptor 1
TAM	Tumor-associated macrophages
TAT	Thrombin-antithrombin
TF	Tissue factor
TGF-α	Transforming growth factor alpha
TIE2	Tyrosine kinase with immunoglobulin-like and EGF-like domains 2
TK-1	Thymidine kinase 1
TME	Tumor microenvironment
TNM	Tumour, node, metastasis
TP53	Tumor protein or transformation-related protein 53
VEGF-A	Vascular endothelial growth factor A
v-SKI	Avian sarcoma viral oncogene homolog
vWF	Von Willebrand factor
Yes-1	Yamaguchi sarcoma virus proto-oncogene tyrosine-protein kinase

Abstract

Canine hemangiosarcoma is a highly aggressive malignant endothelial neoplasm, HSA poses a significant challenge in veterinary oncology due to its aggressive nature and limited treatment options. This literature review begins with a comprehensive overview of key aspects in the field of cancer research, focusing on tumor biology, diagnosis, pathology, cytology, and the intricate mechanisms governing cancer initiation, progression, and metastasis. A central focus of this review lies on elucidating diagnostic approaches for HSA. The limitations and potential of techniques like cytology, histopathology, and hematology are explored. Additionally, the role of emerging biomarkers in facilitating early and accurate diagnosis is investigated. Finally, we evaluate the current understanding of the pathogenesis of HSA and the intricate sequence of events that leads to tumor formation. This includes investigating the role of both genetics and the microenvironment, along with the dysregulation of cellular pathways that fuel uncontrolled proliferation and metastasis.

Keywords : Hemangiosarcoma ; Cytology ; Histopathology ; Microenvironment.

Résumé

L'hémangiosarcome canin est une néoplasie endothéliale maligne très agressive. En raison de sa nature agressive et des options de traitement limitées, le HSA représente un défi important en oncologie vétérinaire. Cette étude bibliographique commence par un aperçu complet des aspects clés dans le domaine de la recherche sur le cancer, en se concentrant sur la biologie des tumeurs, diagnostic, pathologie, cytologie et les mécanismes complexes qui régissent l'initiation, la progression et la métastase du cancer. L'un des principaux objectifs de cette bibliographie est d'élucider les approches diagnostiques du HSA. Les limitations et le potentiel de techniques telles que la cytologie, l'histopathologie et l'hématologie sont explorés. De plus, le rôle des bio-marqueurs émergents pour faciliter un diagnostic précoce et précis est étudié. Enfin, nous évaluons la compréhension actuelle de la pathogénie du HSA et la séquence complexe d'événements qui conduit à la formation de la tumeur. Cela comprend l'étude du rôle de la génétique et du microenvironnement, ainsi que le dérèglement des voies de signalisation cellulaire qui favorisent la prolifération incontrôlée et les métastases.

Mots clés : Hémangiosarcome ; Cytologie ; Histopathologie ; Microenvironnement.

ملخص

ورم الأوعية الدموية الوعائية الكلبية هو ورم بطاني خبيث شديد العدوانية، يشكل تحديًا كبيرًا في طب الأورام البيطري بسبب طبيعته العدوانية وخيارات العلاج المحدودة .تبدأ هذه المراجعة الأدبية باستعراض شامل للجوانب الأساسية في مجال أبحاث السرطان، مع التركيز على بيولوجيا الورم والتشخيص وعلم الأمراض وعلم الخلايا والآليات المعقدة التي تحكم بدء السرطان وتطوره وانتقاله .يتركز محور رئيسي لهذه المراجعة على توضيح الأساليب التشخيصية لورم الأوعية الدموية الوعائية الكلبية. يتم استكشاف قيود وإمكانات تقنيات مثل علم الخلايا وطب الأمراض النسيجية وعلم الدم .بالإضافة إلى ذلك، يتم التحقيق في دور العلامات الحيوية الناشئة في تسهيل التشخيص المبكر والدقيق. أخيرًا، نقوم بتقييم الفهم الحالي لآلية تكون ورم الأوعية الدموية الوعائية الكلبية والترتيب المعقد للأحداث التي تؤدي إلى تكون الورم .يشمل هذا التحقيق دور كل من العوامل الوراثية والبيئة المحيطة، إلى جانب خلل التنظيم في المسارات الخلوية التي تغذي التكاثر والانتقال غير المنضبط.

الكلمات المفتاحية : ورم الأوعية الدموية الوعائية الكلبية ; علم الخلايا ; علم الأمراض النسيجية ; البيئة المحيطة.

Introduction

Hemangiosarcoma, a malignant neoplasm arising from vascular endothelial cells, casts a long shadow over canine health. Its aggressive nature is characterized by rapid growth, a high propensity for metastasis, and a dismal prognosis. Despite its prevalence, the precise origin and mechanisms underlying HSA development remain incompletely understood.

HSA's high mortality rate underscores the need for a deeper understanding of the disease. The median survival time for diagnosed dogs is short, ranging from one to three months with current treatment modalities. The clinical signs are nonspecific until the tumour ruptures and the affected dogs are brought to an emergency unit because of the consequences of internal body cavity blood loss. Early diagnosis remains a significant challenge, often delaying intervention until the disease is already advanced. Immediate euthanasia is therefore commonly chosen by owners and veterinarians.

Despite identifying genomic alterations in HSA cancer cells, the key mutations driving tumor development remain elusive and require further investigation. This presents a significant hurdle in diagnosing HSA. While microscopic examination of tissue samples remains the gold standard, its accuracy can be compromised. Sometimes, the similarity between cancerous and non-cancerous hematoma tissues can lead to misdiagnosis in ambiguous samples. Glycoprotein biomarkers have emerged as potential tools for diagnosing canine HSA. However, their use remains limited due to the lack of extensive validation in separate groups of dogs compared to the initial studies.

While the exact etiology of HSA remains elusive, several factors are suspected to play a role. Genetic predisposition appears to be a contributing element, with certain breeds exhibiting a higher incidence. Additionally, exposure to environmental carcinogens, has been suggested as a potential risk factor. Further research is needed to solidify these associations and identify additional genetic and environmental contributors to HSA development.

The tumor microenvironment is no longer considered a passive bystander; it actively participates in tumor development and progression. Deciphering these intricate relationships within the TME holds immense potential for the development of novel therapeutic strategies targeting not only the tumor cells themselves but also the supportive ecosystem that fuels their growth and spread.

This literature review is divided into chapters that cover a general introduction to oncology, delves into the current understanding of the pathobiology of canine hemangiosarcoma, exploring potential etiological factors, cellular pathways involved in tumorigenesis, and the complex interplay between the tumor microenvironment and metastatic spread.

Chapter I Tumor Biology

1. Introduction

Delineating the biological processes and molecular pathways underlying a disease is a prerequisite for developing successful therapeutic interventions. In veterinary medicine, this principle holds true when battling cancer. Tumor biology, the foundation of veterinary oncology, delves into the complex cellular and molecular mechanisms driving tumor formation, progression, and spread. This field of study encompasses a comprehensive investigation at the cellular and molecular level. It interrogates the genetic and epigenetic alterations that initiate and sustain the neoplastic phenotype, the transformation of a normal cell into a cancerous one (Jones et Baylin, 2007).

Understanding the acquisition of hallmarks of cancer, such as sustained proliferative signaling, evasion of apoptosis, and the ability to invade and metastasize, forms a central pillar of tumor biology research.

Beyond the intrinsic properties of tumor cells, tumor biology recognizes the critical role of the tumor microenvironment. This complex ecosystem, encompassing stromal cells, blood vessels, and the immune system, actively influences tumor behavior. Research in this area explores the intricate interplay between tumor cells and their surroundings, elucidating how tumors manipulate the microenvironment to promote their own growth and evade host defenses (Joyce et Pollard 2008).

The knowledge gleaned from tumor biology research has profound clinical implications. By deciphering the molecular underpinnings of cancer, we pave the way for the development of novel therapeutic strategies. These strategies may target specific signaling pathways within tumor cells themselves, or exploit the tumor microenvironment to enhance anti-tumor immunity or disrupt tumor-promoting interactions (Weinstein et Joe, 2008).

The benefits of tumor biology research extend beyond veterinary medicine. By studying naturally occurring cancers in dogs, cats, and other animals, we can gain valuable insights applicable to human oncology. These discoveries contribute to the development of new diagnostic tools, targeted therapies, and ultimately, improved outcomes for both human and animal cancer patients.

2. Pre-neoplastic changes

Tumor development is a stepwise process. Potential preneoplastic alterations such as dysplasia, metaplasia, hypertrophy, and hyperplasia are therefore important from a diagnostic and therapeutic standpoint. These preneoplastic alterations frequently indicate a higher chance that the afflicted tissue may develop to neoplasia. Hypertrophy is defined as an increase in the size of individual cells through the addition of cytoplasma. Hyperplasia is an increase in the number of cells in a tissue through mitotic division of cells through cellular proliferation. It must be distinguished from hypertrophy, which is an increase in individual cell size through the addition of cytoplasm. Metaplasia, the transformation of one differentiated cell type into another, is most commonly observed in epithelial tissues. Dysplasia is an abnormal pattern of tissue growth and usually refers to disorderly arrangement of cells within the tissue. In general, preneoplastic changes are reversible. They may arise in response to physiological demands, injury, or irritation but often resolve with the removal of the inciting factor. For example, skeletal muscle hypertrophy is an adaptive response to increased workload. The terms hyperplasia and hypertrophy are not appropriate in descriptions of true neoplasms, but the terms dysplasia and metaplasia may describe changes that persist during the transition from preneoplasia to neoplasia. Anaplasia is the term used to describe loss of cellular differentiation and reversion to more primitive cellular morphologic features; anaplasia often indicates irreversible progression to neoplasia (Zachary, 2022).

3. Tumor types

In most neoplasms, microscopic examination reveals a dominant cell type, either of mesenchymal or epithelial origin. This dominant cell lineage guides the tumor's nomenclature, reflecting its presumed cell of origin. However, a subset of tumors arises from pluripotent stem cells, resulting in a mixed tumor phenotype characterized by the presence of multiple distinct cell types and even differentiated tissues within the same neoplasm. Cell of origin identification has been shown to be a strong prognosticator of biologic behavior (Zachary, 2022).

3.1. Mesenchymal tumors

They originate from embryonic mesoderm and exhibit diverse morphologies with spindle or round cell arrangements. Benign tumors are named with the suffix “-oma,” reflecting their cell of origin (e.g., fibroma from fibroblasts). Malignant counterparts are termed "sarcomas," often incorporating a prefix denoting the originating tissue (e.g., fibrosarcoma). Meanwhile malignancies arising from circulating blood cells or their precursors are termed leukemias

(“white blood”) when characterized by large numbers of abnormal hematopoietic cells in the peripheral blood or bone marrow (Zachary, 2022).

3.2. Epithelial tumors

Epithelial tissues, and consequently, the tumors derived from them, can originate from all three primary germ layers: ectoderm, mesoderm, and endoderm. Benign epithelial tumors are categorized based on their morphology and origin. Adenomas represent tumors arising from glandular epithelium, often exhibiting characteristic acinar or ductal structures (e.g., mammary adenomas). Alternatively, adenomas may originate from non-glandular epithelium, displaying a tubular pattern microscopically (e.g., renal tubular adenoma). Papillomas denote benign, exophytic growths typically arising from cutaneous or mucocutaneous surfaces. Polyps are grossly visible, benign epithelial tumors projecting from mucosal surfaces without invading underlying tissues. Although some interchangeability exists, these terms have distinct morphological meanings. Malignant epithelial tumors are classified as carcinomas. Microscopically, these tumors may display nests, cords, or islands of neoplastic epithelial cells. Adenocarcinomas represent a more specific subset exhibiting a distinct glandular growth pattern, similar to the normal glandular epithelium. Distinguishing between adenocarcinomas with acini (e.g., salivary) and those with tubular differentiation (e.g., renal) based solely on morphology can be challenging. By definition, all carcinomas are invasive and have the potential to metastasize. Additionally, these terms are frequently modified by prefixes or adjectives reflecting their microscopic characteristics. For instance, the term "squamous cell carcinoma" denotes an epithelial neoplasm with differentiation resembling normal stratified squamous epithelium (Zachary, 2022).

3.3. Tumors of neural crest cells

Neural crest cells, distinguished from both mesenchymal and epithelial lineages, give rise to a diverse array of specialized cell types. Unlike sarcomas (mesenchymal) and carcinomas (epithelial), which adhere to distinct ontogenetic classifications, neural crest tumors require a separate nomenclature. Benign tumors arising from neural crest cells retain their specific cell type designation, appended with the "-oma" suffix reflecting their differentiated state. For example, benign tumors of the adrenal medulla are known as pheochromocytomas. However, their malignant counterparts deviate from the typical "sarcoma/carcinoma" convention. Instead, they are simply designated as "malignant pheochromocytoma," incorporating the "malignant" descriptor within the existing terminology (Zachary, 2022).

Tissue or Cell of Origin	Benign	Malignant
Epithelial		
Squamous	Squamous papilloma	Squamous cell carcinoma
Transitional	Papilloma	Transitional cell carcinoma
Glandular	Adenoma, cystadenoma	Adenocarcinoma, cystadenocarcinoma
Mesenchymal		
Fibrous tissue	Fibroma	Fibrosarcoma
Adipose tissue	Lipoma, infiltrative lipoma	Liposarcoma
Cartilage	Chondroma	Chondrosarcoma
Bone	Osteoma	Osteosarcoma, multilobular osteochondrosarcoma
Muscle (smooth)	Leiomyoma	Leiomyosarcoma
Muscle (striated/skeletal)	Rhabdomyoma	Rhabdomyosarcoma
Endothelial cells, blood vasculature	Hemangioma	Hemangiosarcoma
Endothelial cells, lymphatic vasculature	Lymphangioma	Lymphangiosarcoma
Synovium	Villonodular hyperplasia (nonneoplastic)	Synovial cell sarcoma
Mesothelium	-	Mesothelioma
Melanocytes	Benign melanoma (melanocytoma)	Malignant melanoma, Melanosarcoma
Peripheral nerve	-	Malignant schwannoma, neurofibrosarcoma, peripheral nerve sheath tumor
Perivascular wall	-	Perivascular wall tumor (PVWT) (previously hemangiopericytoma)
Uncertain origin	-	Malignant fibrous histiocytoma (MFH)
Hematopoietic and Lymphoreticular		
Lymphocytes	-	Lymphoma (tissue involvement) with subclassifications and leukemic (in circulation) forms
Plasma cells	Cutaneous plasmacytoma	Multiple myeloma, plasmacytoid or plasmablastic lymphoma
Granulocytes	-	Myeloid leukemia
Red blood cells	-	Erythroid leukemia
Platelets	-	Megakaryocytic or megakaryoblastic leukemia
Histiocytes (macrophages or dendritic cells)	Histiocytoma	Histiocytic sarcoma, malignant histiocytosis
Mast cells	-	Mast cell tumor
Thymus	Thymoma, noninvasive	Malignant thymoma (invasive), thymic carcinoma

Neural		
Glial cells	Astrocytoma, oligodendroglioma	Astrocytoma, glioblastoma multiforme, oligodendroglioma
Meninges	Meningioma	Malignant meningioma
Gonadal		
Germ cells	Seminoma, dysgerminoma	Seminoma, Dysgerminoma
Supportive cells	Sertoli cell tumor, granulosa cell tumor	Sertoli cell tumor, granulosa cell tumor
Interstitial cells	Interstitial (Leydig) cell tumor, thecoma, luteoma	Interstitial (Leydig) cell tumor

Table 1. Nomenclature of common tumor types in veterinary medicine (Vail, 2020).

4. Mechanism of carcinogenesis and genetic changes

4.1. Oncogenes

These genes in their native state typically hold critical roles in regulating cell growth and proliferation, but when activated by overexpression or mutation, in this case proto-oncogenes are termed oncogenes, they unleashe a cascade of dysregulation that drives cell proliferation and render the cell unresponsive to normal growth inhibitory signals, ultimately resulting in tumor formation.

4.1.1. Growth factors

Growth factors (GFs) are signaling molecules that interact with specific cell surface receptors, modulating cellular processes such as proliferation, and differentiation However, their involvement in carcinogenesis can occur through excessive production of the GF or through ectopic expression in a cell type that does not normally express that GF (Morris, 2001).

4.1.2. Growth factor receptors

These proteins are integral components of cell surface receptors, acting as the initial point of contact for GF ligands. Alterations within these proto-oncogene-derived proteins can play a pivotal role in carcinogenesis (Morris, 2001).

4.1.3. Protein kinases

They are located on the inner plasma membrane, play a crucial role in signal transduction following ligand-receptor interaction. Structural alterations in these genes and their encoded proteins can lead to enhanced kinase activity. This dysregulation has profound effects on downstream signaling pathways. One key element of this signaling cascade is the sequential phosphorylation of signaling intermediaries, including second messengers like GTP and GTP-binding proteins (G-proteins). During signal transduction, GTP is converted to GDP by the

intrinsic GTPase activity of G-proteins. A group of proto-oncogenes known as Ras encode proteins with both GTPase activity and the ability to bind GTP. In normal cells, these proteins function as modulators of cellular proliferation. However, mutations in the Ras proto-oncogene can lead to uncontrolled cell proliferation (Morris, 2001).

4.1.4. Nuclear proteins and transcription factors

They are crucial proteins that govern the expression of other genes. These genes often play critical roles in cellular proliferation, making them potential instigators of cancer development. Unsurprisingly, alterations in the activity of these transcription factors are implicated in the emergence of the malignant genotype.

There are numerous ways in which proto-oncogenes can be activated:

- Chromosomal translocation: when proto-oncogenes are translocated within the genome (i.e., from one chromosome to another) (Morris, 2001).

- Gene amplification: a gene can be amplified so that a signal to transcribe the gene results in the production of many more copies of mRNA than usual amplification of oncogenes can occur in a number of tumor types and has been demonstrated in juvenile canine soft tissue sarcomas, in which the *MDM2* proto-oncogene is was shown to be amplified numerous times, gene amplification is perhaps the most common mechanism of proto-oncogene activation (Morris, 2001).

- Point mutations: these are single base changes in the DNA sequence of proto-oncogenes, leading to the production of abnormal proteins. A mutation in a proto-oncogene or the transcriptional machinery that controls its expression may disrupt homeostasis and result in sustained proliferation signals or failure to respond to negative feedback signals (Morris, 2001).

- Viral insertions: in some circumstances proto-oncogene function can be damaged by the insertion of viral elements. Occasionally, novel retroviruses are isolated from leukemias or sarcomas in animals that have been viremic with a leukemia virus for some time. The prototype of the acutely transforming virus is the Rous sarcoma virus (RSV). Subsequently, many more have been isolated from animals infected with avian, feline, murine, or simian oncoviruses. These viruses are generated by a rare recombinant event between the leukemia virus, with which the animal was originally infected, and a cellular proto-oncogene. In this, part of the viral genome is deleted and replaced with the cellular oncogene. The virus then becomes acutely transforming because this oncogene is now under the transcriptional control of a very efficient

viral promoter. This then allows infection of a cell and insertion of this continuously expressed oncogene into the cellular genome. Evidence suggests that these acutely transforming viruses are not transmitted naturally, but all events occur in the individual animal. Because the virus has itself lost some of its own genetic material, it is defective for replication; however, it is spread throughout an animal by the provision of help from the normal leukemia virus, which provides the missing proteins in co-infected cells. In addition to acutely transforming mechanisms, retroviruses can activate cellular oncogenes by integrating adjacent to them. A good example of this is the *myc* gene, which is frequently activated in feline T-cell lymphomas (Vail, 2022).

Virus	Tumour	Species
DNA viruses		
Adenovirus family	Adenomas	Sheep
Mareks disease virus	Mareks disease/lymphoma	Chicken
Papovavirus family	Papillomas/warts	Man and animals
RNA viruses Retrovirus family		
BLV	Bovine leukosis/lymphoma	Cow
ALV	Avian leukosis/lymphoma	Chicken
FeLV	Leukaemia/lymphoma	Cat

Table 2. Example of some tumor-causing viruses (Morris, 2001).

4.2. Tumor suppressor genes

In contrast to the stimulatory effects are provided by the proto-oncogenes, as described earlier, tumor formation can result from a loss of inhibitory functions associated with another class of cellular genes, Tumor Suppressor Genes. Originally the name was given to genes that inhibited cell proliferation. Over time the class of tumor suppressor genes has expanded to include many different types of cancer-related genes that, when inactivated through genetic or epigenetic means, allow uncontrolled cell proliferation and tumor growth. Suppressor genes include genes that control cell cycle, apoptosis, DNA repair, and other fundamental pathways (Morris, 2001).

4.2.1. The *p53* tumor suppressor gene

p53 protein, depending on the severity of DNA damage, it can either push cells into temporary arrest for repair or apoptosis This pivotal role extends beyond immediate action, as *p53* also regulates gene expression for DNA repair, differentiation, and the overall cellular response to damage. Through these mechanisms, *p53* acts as a potent tumor suppressor, preventing the accumulation of potentially cancerous mutations and maintaining genomic stability. However, when *p53* function falters, the result is abnormal uncontrolled cell growth. This dysfunction can occur through various pathways, gene mutations: nonsense, missense, and splice site mutations,

along with allelic loss, rearrangements, and deletions, directly impair *p53* activity, or non-mutational mechanisms: nuclear exclusion, complex formation with viral proteins, and overexpression of the *MDM2* oncogene can also disrupt *p53* function without altering the gene itself (Meuten, 2017).

Altered gene	Species	Tumour types	Reference
p53	Dog	Osteosarcoma	Sagartz et al., 1996
		Squamous cell carcinoma	Gamblin et al., 1997
		Nasal adenocarcinoma	Mayr et al., 1998a, 1999
		Peri-anal gland adenocarcinoma	Veldhoen et al., 1999
		Mammary adenoma/carcinoma	Nasir & Argyle 1999
		Lymphoma	
K-ras	Dog	Lung carcinoma	Kraegel et al., 1992
N-ras	Dog	Acute non-lymphocytic leukaemia	Gumerlock et al., 1989
yes-1	Dog	Mammary and other tumors	Miyoshi et al., 1991b Rungsipipat et al., 1999
myc	Dog	Plasma cell tumours	Frazier et al., 1993

Table 3. Molecular changes in dog and cat tumors (Morris, 2001).

4.3. Multistep carcinogenesis

The development of neoplasms, is a complex and protracted process driven by the accumulation of numerous genetic and epigenetic alterations over time. These modifications progressively disrupt cellular regulatory pathways, ultimately leading to the emergence of a fully transformed tumor cell. This gradual progression, characterized by the sequential acquisition of specific mutations, is aptly described as stepwise tumor development.

4.3.1. Initiation

The initial stage of carcinogenesis, initiation, marks an irreversible turning point. This pivotal step involves the introduction of a persistent genetic alteration within normal cells, orchestrated by mutagenic initiating agents or initiators. These agents, encompassing both chemical and physical carcinogens, inflict DNA damage, laying the groundwork for potential tumor development. The presence of a DNA lesion alone is insufficient for mutation induction. This process necessitates misrepair of the lesion during subsequent DNA replication, resulting in a permanent alteration within the complementary DNA strand. This underscores the importance of at least one replication cycle for the genetic change to become firmly established and potentially drive tumorigenesis. Initiated cells initially exhibit seemingly normal morphology and may even remain quiescent for extended periods. However, these seemingly unaltered cells harbor "dormant" mutations that could confer a growth advantage under specific conditions. This advantage could manifest in various ways, such as an enhanced response to growth-promoting signals or increased resistance to apoptosis (Zachary, 2022).

4.3.2. Promotion

The second stage of tumor development, promotion, witnesses the outgrowth of initiated cells in response to specific selective stimuli. These stimuli, termed promoting agents or promoters, primarily drive cellular proliferation. Notably, promoters generally lack mutagenic activity; instead, they foster a proliferative environment that grants initiated cells a growth advantage. With every subsequent cell division, these cells become increasingly susceptible to acquiring additional mutations, potentially furthering tumor progression. The culmination of the promotion phase often manifests as a benign tumor (Zachary, 2022).

4.3.3. Progression

The final stage of tumor development, progression, marks the transition from a benign tumor to a progressively malignant entity through malignant transformation. This irreversible shift enables the tumor to metastasize and disseminate throughout the body. The precise mechanisms underlying progression is believed to be a complex interplay of genetic and epigenetic alterations within tumor cells, coupled with changes to the tumor microenvironment that favor the selection of increasingly malignant tumor cell clones. According to the latest established cancer hallmarks, tumor progression is marked by genetic instability in tumor cells and increasing tumor cell heterogeneity (Zachary, 2022).

4.4. Clonal selection

The prevailing theory suggests most tumors descend from a single transformed cell that has undergone oncogenic changes. During tumor growth, progressive accumulation of heritable mutations within these transformed cells drives the generation of tumor heterogeneity. Each new mutation creates a subclone of tumor cells, progeny of the original mutated cell. This subcloning process is amplified by the significantly higher genomic instability of tumor cells compared to their normal counterparts. Successful subclones emerge through selection pressure, favoring those with specific advantageous traits. These include: enhanced proliferation, immune evasion, angiogenesis, and exogenous growth factor independence. These characteristics confer a selective advantage to successful subclones, allowing them to outcompete and eventually dominate the tumor population. This process is referred to as clonal selection (Zachary, 2022).

5. Angiogenesis

Solid neoplasms rely on the recruitment of blood vessels and supportive stroma from surrounding tissues for their continued growth. Without vascularization, tumor masses are

physically limited to approximately 1-2 mm in diameter. Angiogenesis, normally tightly regulated by pro- and anti-angiogenic signals, undergoes an imbalance in favor of vessel growth within neoplastic environments. Tumors disrupt this balance by secreting growth factors s such as vascular endothelial growth factor A (VEGF-A) and various types of fibroblastic growth factors (FGF) (e.g. PlGF and bFGF). This shift in the angiogenic landscape within the tumor microenvironment is primarily driven by tumor cells themselves. They achieve this by:

- Directly secreting potent growth factors, such as vascular endothelial growth factor A (VEGF-A) and various fibroblastic growth factors (FGFs) (e.g., PlGF and bFGF). These factors directly stimulate the proliferation and migration of endothelial cells, the building blocks of blood vessels.

- Indirectly inducing the release of angiogenic factors from other cell types within the tumor microenvironment. This includes adjacent stromal elements, bone marrow-derived immune cells (macrophages, neutrophils, and mast cells), and even myeloid precursors infiltrating the tumor margins.

Researchs have shown that angiogenesis initiates during the early stages of tumorigenesis, evident even in preneoplastic and benign lesions. Similarities do exist between the processes of angiogenesis and stroma formation within the contexts of tumor progression and wound healing. This has fostered the insightful concept of tumors as "nonhealing wounds." In tumors, blood vessels display poor differentiation and heterogeneous distribution throughout the mass. Characterized by tortuosity, dilation, and persistent endothelial gaps, tumor vasculature exhibits elevated permeability compared to transient permeability within vessels associated with wound healing. Given that tumor cell entry into the bloodstream primarily occurs through these endothelial gaps, this abnormal vasculature likely facilitates metastatic spread. Recruited endothelial cells contribute to beyond providing essential perfusion they also promote tumor progression through the secretion of growth factors that directly stimulate tumor cell proliferation. Notably, the presence of angiogenesis alone does not definitively indicate malignancy, as benign neoplasms also possess the ability to induce vascular growth. Tumor stroma, a complex microenvironment encompassing non-neoplastic connective tissue, blood vessels, and inflammatory cells, provides crucial support for tumor development and progression. While the vasculature is undeniably essential for nutrient delivery, the non-vascular component constitutes the majority of the tumor stroma. It is noteworthy that, carcinoma-associated fibroblasts (CAFs), a specific type of fibroblast or myofibroblast found adjacent to carcinomas, display a distinct phenotype compared to fibroblasts in other tissues.

These CAFs exhibit fetal-like characteristics and, in response to signals from neoplastic epithelial cells, can facilitate tumor cell proliferation through the release of growth factors and proteases. Furthermore, CAFs contribute significantly to the processes of angiogenesis, invasion, and ultimately, metastasis (Straw, 2009)

6. Mechanisms of tumor dissemination and metastasis

6.1. Detachment

As a starting point in invasion and metastasis, tumor cells must escape the main tumor mass, penetrate the basement membrane, and enter the ECM. For cells to separate from each other, they sever intercellular bonds, including desmosomes and adherens junctions. In many tumor cells of epithelial origin, this process is due to loss of cadherins or catenins. Next, they build bridges to the surrounding matrix. Integrins and other specific receptors on tumor cell membranes recognize and bind to a variety of ECM components such as fibronectin, laminin, collagen, and vitronectin. During invasion and metastasis tumor cells can dynamically adjust their surface receptors to navigate different microenvironments (Zachary, 2022).

6.2. Migration

At many points during invasion and metastasis, tumor cells migrate actively. Their migration is guided by autocrine growth factors, such as hepatocyte growth factor (HGF) and by cleavage products of ECM components, including fragments of collagen. This migration is facilitated by alterations in the cytoskeleton and in cellular adhesion structures to which the cytoskeletal components are anchored (Zachary, 2022).

6.3. Stromal invasion

In malignant tumors, the neoplastic epithelial cells actively dismantle basement membrane and ECM components by increasing net protease activity, paving the way for invasion. This activity results from a complex interplay of interacting factors, including the rate of protease synthesis and activation and the rate at which protease inhibitors are produced. Proteases and antiproteases may be produced and activated by the tumor cells themselves, or tumor cells may induce nonneoplastic stromal cells to produce these enzymes. Key players in this process include matrix metalloproteinases (MMPs), such as type IV collagenase, and urokinase, a serine protease (Zachary, 2022).

6.4. Epithelial-mesenchymal transition

some carcinomas undergo a dramatic metamorphosis called epithelial-mesenchymal transition (EMT). This switch sees them abandon their tightly bound epithelial identity for a more free-

roaming mesenchymal state. The transformation is marked by a loss of intercellular adhesion, a surge in migration-promoting proteases, acquisition of motility, and a shift from epithelial markers like cytokeratin to mesenchymal vimentin. These once-immobile epithelial cells morph into spindle-shaped, fibroblast-like entities, gaining the ability to infiltrate surrounding tissues. Driving this shift are transcription factors, conducting the expression of genes involved in adhesion, migration activation, and protease production (Zachary, 2022).

6.5. Intravasation

The process of tumor cells breaching the vascular barrier and entering the bloodstream or lymphatic system, known as intravasation. Tumor cells are attracted to vessels by chemotactic factors produced by multiple cell types and migrate through the ECM with the aid of tumor-derived proteases. process is often aided by tumor-associated macrophages, immune cells that accompany and support tumor invasion. Additionally, the naturally increased permeability of newly formed blood vessels in tumors can further facilitate intravasation (Zachary, 2022).

6.6. Tumor emboli

Once inside a lymphatic or blood vessel, tumor cells exhibit a propensity to aggregate, forming small emboli. These clusters are held together by shared adhesion molecules, facilitating their collective movement through the vascular system. However, their presence within vessels exposes them to host lymphocytes that can recognize and attack them. Interestingly, platelets can also interact with tumor emboli. While this interaction might initially seem detrimental, evidence suggests that platelets can shield the embolus from immune-mediated destruction (Zachary, 2022).

6.7. Extravasation

This process involves tumor cells leaving the blood or lymphatic vessels, it’s primarily determined by their ability to interact with specific adhesion molecules on endothelial cells lining the vessels. Upon attachment to the endothelium, tumor cells pass between or through endothelial cells and penetrate the basement membrane to enter the ECM, thus establishing a metastatic site. The newly formed metastatic site must offer a suitable microenvironment for tumor cell growth and survival. Without such support, these cells may struggle to thrive, potentially remaining dormant or being eliminated by the immune system (Zachary, 2022).

7. Immune system evasion

7.1. Altered major histocompatibility complex expression

Cytotoxic T lymphocytes recognize tumor antigens only when presented on the surface of tumor cells alongside MHC class I molecules. Thus, the Loss of MHC class I will allow them to evade CTL detection but also exposes them to attack by natural killer (NK) cells. Tumors may also downregulate expression of class II MHC antigens. Class II antigens are required for activation of TH lymphocytes that stimulate CTL differentiation, and loss of these antigens may hinder the development of a strong CTL response (Meuten, 2017).

7.2. Antigen masking

Tumors may become invisible to the immune system by losing or masking their tumor antigens. Tumor evolution favors the outgrowth of variants that lack or downregulate their unique tumor antigens, making them indistinguishable from healthy cells. Tumor can be hidden behind a cloak of sugars (glycocalyx), or fibrin. This cloak can render them invisible to the immune system, particularly cytotoxic T lymphocytes. Some antibody responses to tumor antigens might actually be counterproductive, by binding to the antigens, they can create a protective shield, preventing CTLs from recognizing the tumor cells (Meuten, 2017).

7.3. Tolerance

Tolerance is inability to mount an immune response against a specific antigen, this phenomenon manifests in multiple ways: the immune system is naturally programmed to tolerate self-antigens, hindering responses to tumor antigens shared with normal tissues, even non-self antigens like tumor antigens can be rendered "invisible" to the immune system if presented without essential co-stimulatory molecules needed for T cell activation, or through immunosuppression driven by T reg cells present within tumors that actively promote tolerance to tumor antigens (Meuten, 2017).

7.4. Immunosuppression

Neoplastic cells may possess an arsenal of immunosuppressive factors that help them evade immunosurveillance if released. Many tumors produce TGF-α, which inhibits the proliferation and function of lymphocytes and macrophages. Tumors may also produce and express Fas ligand a molecule that triggers apoptosis in T lymphocytes upon binding to their Fas receptors, effectively silencing immune cell. In addition, tumor cells may release tumor-derived antigens into the circulation that form immune complexes with antibodies. While these

complexes aim to neutralize antigens, they can paradoxically suppress immune function in some cases (Meuten, 2017).

Chapter II Tumor Diagnosis

1. Introduction

Accurately diagnosing tumors is the cornerstone of effective cancer management in animals. Veterinary medicine has witnessed significant advancements in diagnostic techniques, enablinga more precise and timely identification of malignancies in pets. Early and accurate diagnosis plays a critical role in determining the most appropriate treatment course, ultimately improving patient outcomes and quality of life.

This diverse field encompasses various diagnostic modalities, each offering unique advantages and limitations. Cytology, the microscopic examination of cells obtained through techniques like fine-needle aspirates, provides a minimally invasive and rapid approach for initial evaluation of suspicious masses. Histopathology, the examination of tissue samples under a microscope, remains the gold standard for definitive tumor diagnosis, offering detailed information about the type, grade, and other characteristics of the cancer. Additionally, advanced diagnostic tools like immunohistochemistry utilize specific antibodies to identify tumor markers, further aiding in diagnosis and treatment planning.

The choice of diagnostic approach often depends on the location, size, and accessibility of the tumor. Collaboration between veterinarians and pathologists is crucial throughout the diagnostic process. Accurate diagnosis lays the foundation for developing a personalized treatment plan tailored to the specific type and stage of the cancer, ultimately influencing the pet's prognosis and potential for successful management.

The initial evaluation aims to achieve several key objectives:

- To confirm the existence of cancer
- To grade the disease
- To determine the extent of the disease in terms of both local and distant spread
- To investigate and address any tumor-related or concurrent complications that might make treatment harder or less effective for the patient (Morris, 2001).

2. Physical exam

Thorough physical examination can offer an important tool for early detection of neoplasia, in many cases, give a good outcome. Thorough examination of lumps and bumps via fine needle aspirates (FNAs) can lead to the early detection of tumors. Such tumors are much easier to remove when small and therefore will have potentially a better outcome for the individual. The

key to improving prognosis for many veterinary patients is both early detection and immediate action. Abnormalities on physical examination should always be followed up. If an external lump is found on examination always palpate the draining lymph node for signs of enlargement; this is the beginning of the important process of staging (Sivaseelan, 2021).

Physical examination can provide useful information about :

- Tumour site and relationship to normal anatomic structures
- Size or volume of the primary mass
- Mobility of the tumour with respect to surrounding tissues: fixation usually denotes tumour infiltration of adjacent structures
- Ulceration denotes infiltration and disruption of the epidermis (Kiehl, 2016).

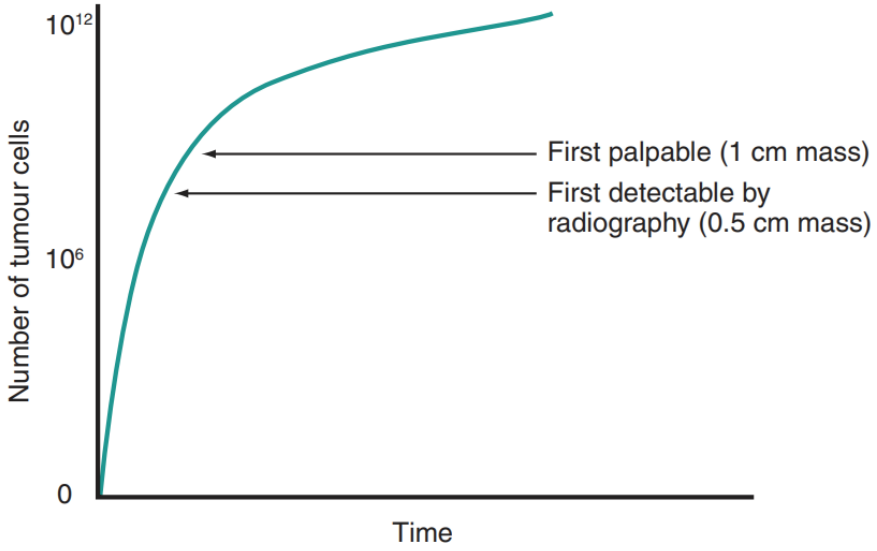

Figure 1. A tumor cannot be detected by palpation, radiography or other imaging techniques until it reaches approximately 0.5–1 cm in diameter or 0.5–1 g in weight, by which time it contains approximately 10^8 –10^9 cells (Dobson, 2016).

3. Diagnostic imaging

3.1. Radiography

While conventional radiography has long been the go-to tool for assessing cancer patients due to its affordability and wide availability, newer modalities with greater sensitivity and specificity are gaining ground. However, radiography remains especially useful for:

- Tumors involving or adjacent to bone (e.g. tumors of the oral cavity)

- Tumors sited within body cavities, contrast studies may be required to assist visualization of tumors sited within hollow organs (Morris, 2001).

3.1. Ultrasonography

Ultrasound is very good at identifying and pinpointing lesions within soft tissues and internal organs like the liver, spleen, and kidneys, but the sonographic appearance doesn't definitively reveal the specific nature of these lesions (Morris, 2001).

3.2. Endoscopy

Endoscopy allows visual examination of the respiratory, gastro-intestinal and urogenital tracts (Morris, 2001).

3.3. CT/MRI

CT stands out as the preferred imaging technique for evaluating pulmonary metastases, offering a detailed view of potential cancer spread to the lungs. Beyond the lungs, it also provides valuable information about bone lesions, including the size and the involvement of bone in soft tissue tumors adjacent to bone. MRI allows better evaluation of soft tissues and brain than CT, but the availability of both CT and MRI is limited (Morris, 2001).

4. Pathology

4.1. Cytology

Fine-needle aspirates or impression smears of solid tumors, as well as the cytological examination of cellular contents obtained from organs or body cavities, serve as valuable sources of information regarding lesions. In most instances, such analyses facilitate the differentiation between inflammatory and neoplastic processes. The morphological characteristics of neoplastic cells, in turn, often offer insights into the probable nature of a tumor and its level of malignancy. Given the inherent limitations of cytological assessments, particularly in the absence of the ability to evaluate tissue architecture, the cytologic estimation of tumor malignancy is primarily confined to the morphologic atypia in cells. These atypical features are referred to as cytologic criteria of malignancy and encompass attributes such as anisocytosis, anisokaryosis, macrocytosis, macrokaryosis, and the presence of bizarre mitotic figures. Additionally, features like binucleation, multinucleation, prominent nucleoli, and open chromatin are considered indicative of malignancy, albeit contingent upon their abnormality within the context of the tissue type. The strength of these malignancy criteria is heightened when accompanied by simultaneous variations in size, shape, or number of nuclei or nucleoli within the same cell, denoted as intracellular anisokaryosis or anisonucleosis. These criteria are predicated on the assumption that nonmalignant cells typically exhibit uniformity in cell and nuclear size, transcribe less DNA, and consequently have fewer or less prominent nucleoli.

Malignant cells, in contrast, exhibit greater variability in appearance, often possessing larger nuclei, more open chromatin, and visible nucleoli. However, the presence of these features is not absolute, and some may even occur in non-malignant cells, particularly in metabolically active (hepatocytes) or differentiating tissues. Further complicating the picture, some tumors, like neuroendocrine ones, might lack classic features of malignancy. Furthermore, these criteria might not apply to lymphoid tissues due to their inherent heterogeneity. Hence, the application of these criteria is less straightforward in solid lymphoid neoplasms. Therefore, cytological features offer only an estimate of malignancy, requiring interpretation alongside clinical, anatomical, imaging, and tumor-specific knowledge (Dobson, 2016 ; Burton, 2024 ; Sivaseelan, 2021 ; Vail, 2020).

4.2. Histology

While cytology offers valuable insights, the gold standard for definitive cancer diagnosis remains histological examination of representative tumor tissue. Through a biopsy, the pathologist gains access to the tumor's cellular components, architecture, and relationship with surrounding normal tissues. This comprehensive analysis allows for the identification of malignant features, including abnormal morphology, high mitotic activity, unusual mitoses, high nuclear-to-cytoplasmic ratio, and evidence of invasion or metastasis. Additionally, the cell of origin and degree of differentiation are assessed, sometimes aided by identifying specific cellular products like osteoid in osteosarcomas. Notably, many malignant tumors lack complete differentiation, making origin determination challenging. In such cases, immunohistochemistry utilizes specific cell markers to assist in diagnosis. In essence, while cytology provides valuable clues (Vail, 2020 ; Zachary, 2020).

Cytological features	
Cell population	Pleomorphism Presence of mitoses, especially abnormal or bizarre forms
Cellular features	Large cell size/giant cells (anisocytosis) Poorly differentiated, anaplastic cells High nuclear to cytoplasmic ratio
Nuclear features	Large nuclear size, nuclear pleomorphism (anisokaryosis) Multiple nuclei (often of variable size) Hyperchromatic nuclei with clumping or stippling of chromatin Prominent and often multiple nucleoli of variable size and shape
Histological features	

Cellular features	As outlined for ‘cytology’
Tumour architecture	Lack of structural organisation of cells into recognisable form
Relationship with adjacent tissues	Invasion of cells into adjacent normal tissues
Evidence of metastatic behaviour	Tumour cells invading or present within lymphatics or venules

Table 4. Cytological and histological features of malignancy (Morris, 2001).

4.3. Immunohistochemistry

IHC serves as a valuable tool for confirming a histological diagnosis established through standard stained sections and for determining the cell of origin in poorly differentiated tumors. In addition, it can be used to aid in tumor prognosis. The technique involves the detection of antigen-specific antibodies binding to their antigen targets (e.g. CD79a a marker of B-cell origin) in a tissue section. An insoluble colored precipitate forms at the antigen–antibody binding site and is visualized using a microscope. The extremely selective nature of antibody binding to its ligand is utilized to identify the extent and cellular localization of expression of the antibody, and its target antigen within a tissue. The specific immunohistochemical diagnosis holds substantial prognostic significance, providing pivotal information for clinical outcomes. Nevertheless, the identification of distinct features within subtypes of the same tumor, such as distinguishing between low-grade and high-grade variants, can also serve as a valuable guide for therapeutic decisions and prognostic predictions. The refinement of tumor grading can be enhanced through the evaluation of various additional markers, such as by measurement of proliferation markers (Dobson, 2016).

- Argyrophilic nucleolar organizing regions (AgNORs) are loops of DNA that contain ribosomal genes, and it is the NOR-associated proteins that bind the silver molecules of the stain. An increase in the number of AgNOR proteins indicates an increased demand for ribosomal biogenesis and therefore a higher metabolic activity. High AgNOR counts are associated with a shorter cell cycle and therefore a higher rate of cell proliferation. In companion animal species, they are predictive of survival times in canine lymphoma and canine MCTs (Dobson, 2016).

- Ki67 is an unidentified antigen to which the monoclonal antibody MIB-1 binds. Ki67 is a very large nuclear protein that is expressed exclusively by cells during the cell cycle. Ki67 has been shown to be an independent prognostic factor in canine MCTs. In general, the greater the proliferation index of a mast cell tumour, the more likely it is to recur locally and to metastasize (Dobson, 2016).

5. Staging

The stage of a cancer is often confused with the grade of a cancer, the grade of a tumor indicates how closely it resembles the tissue from which it is derived, meanwhile staging paints a broader picture, it's a clinical assessment and quantifies various parameters, such as the tumor's location within the body, its size, and the presence of local lymphatic or distant metastasis. Staging is primarily conducted by clinicians, who assess and compile information to determine the extent of the cancer's spread. Pathologists can contribute to this process by analyzing adjacent stromal or lymphatic tissue submitted for examination. Documentation of lymphatic or vascular invasion in biopsy tissues is particularly valuable information that pathologists can provide, aiding clinicians in accurately staging the cancer and informing decisions regarding the appropriate course of treatment (Dobson, 2016 ; Sivaseelan, 2021).

The World Health Organization's TNM Classification of Tumors in Domestic Animals stands as one of the extensively utilized and adapted systems in veterinary medicine. While the direct application of TNM staging nomenclature may not be deemed necessary for everyday veterinary practice, the fundamental concept of assessing the tumor's extent in terms of local invasion, as well as nodal and distant metastasis, remains crucial in the comprehensive evaluation of cancer cases.

Stage	Characteristics
T – Primary tumour	
T0	No evidence of tumour
T1	Tumor less than 5 cm diameter and confined to primary tissues
T2	Tumor 5 cm or greater or ruptured, invading subcutaneous tissues
T3	Tumor invading adjacent structures, including muscle
N – Regional lymph nodes	
N0	No regional lymph node (RLN) involvement
N1	Involvement of regional lymph nodes
N2	Distant lymph node involvement
M – Distant metastasis	
M0	No distant metastasis
M1	Presence of distant metastases
Stage	Tumour, node, metastasis
I	T0 or T1; N0; M0
II	T1 or T2, N0 or N1, M0
III	T2 or T3, N0, N1 or N2, M1

Table 5. Example of a proposed clinical staging model (TNM) for canine cutaneous HSA ; Adapted from Mullin et Clifford (Vail, 2020).

Chapter III Clinical and Pathological Aspects of Hemangiosarcoma

1. Introduction

Canine hemangiosarcoma presents a significant challenge within the field of veterinary oncology. This highly aggressive tumor arises from the malignant transformation of endothelial cells lining the vascular system and/or possibly from hemangioblasts or angioblasts, which are early-stage precursor cells capable of differentiating into both blood and endothelial cell, with a particular propensity for the spleen and heart. Its insidious nature, often characterized by a rapid and unpredictable course, necessitates a comprehensive understanding of its biological and clinical aspects.

Unlike many malignancies that exhibit a gradual progression, canine hemangiosarcoma frequently presents with a cryptic clinical picture. Early signs can be vague and nonspecific, encompassing lethargy, weakness, abdominal distention, or mucous membrane pallor. The ambiguity of these clinical manifestations can lead to misdiagnosis and a delay in the initiation of appropriate therapeutic interventions.

Digging deeper into canine hemangiosarcoma necessitates exploring the potential etiological factors and risk factors contributing to its development. Furthermore, a thorough examination of the various anatomical presentations of the disease is crucial, as the affected organ system can significantly influence clinical signs and prognosis. Additionally, elucidating the diagnostic modalities employed for definitive diagnosis and the available treatment options holds paramount importance in effectively managing this complex disease.

By fostering a deeper understanding of canine hemangiosarcoma, we can enhance our ability to identify subtle clinical presentations, potentially leading to earlier diagnosis and improved outcomes. This knowledge empowers both veterinary professionals and pet owners to navigate the complexities of this aggressive malignancy.

2. Nomenclature

Hemangiosacoma is the established term for malignant tumors arising from the lining of blood vessels. Human medicine uses angiosarcoma, encompassing both blood and lymphatic vessel tumors, and often interchangeably with HSA. Additionally, hemangioendothelioma refers to a benign vascular tumor with features blending benign and malignant characteristics. In rare instances, "malignant hemangioendothelioma" might be used, but usually signifies HAS (Oungsakul, 2020)

3. Epidemiology

Hemangiosarcoma exhibits a marked species predilection, occurring predominantly in dogs. Within the canine population, HSA accounts for approximately 2% of all tumors and can be particularly aggressive, comprising 45-51% of splenic malignancies. Conversely, feline HSA is significantly less frequent, observed in only approximately 0.5% of necropsied cats and representing 2% of feline neoplasms overall. While HSA can occur at any age, it is primarily diagnosed in middle-aged to older animals, with rare cases reported in dogs younger than 3 years. No breed is immune, yet specific breeds demonstrate a higher incidence, including German Shepherds, Golden Retrievers, Labrador Retrievers, and other large-sized dogs. Males might exhibit a slight predisposition to HSA in the canine population. Additionally, studies hint at a possible hormonal influence, with spaying and late neutering in females potentially being associated with increased HSA risk. Interestingly, in dogs, cutaneous HSA is more prevalent in areas exposed to ultraviolet radiation (e.g., ventral abdomen), especially in short-haired and lightly pigmented breeds, suggesting a potential role of UV exposure (Witter, 2015 ; Meuten, 2017)

4. Clinical Signs

Clinical presentation of hemangiosarcoma (HSA) is highly dependent on tumor location and ranges from nonspecific signs to acute collapse and death due to hemorrhagic shock. Most cases of visceral HSA necessitate emergency intervention following tumor rupture and internal bleeding, leading to lethargy, weakness, collapse, weight loss, reduced appetite, abdominal distension, vomiting, exercise intolerance, and difficulty breathing. Renal HSA may present with a history of hematuria. Physical examination in the emergency setting can reveal tachycardia, weak pulse, pale mucous membranes, and signs of fluid accumulation in the abdomen or an abdominal mass. Cardiac tamponade due to right atrial HSA rupture presents critically, with muffled heart sounds, pulsus paradoxus, ascites, or circulatory collapse. Cutaneous or subcutaneous HSA may manifest as small, blood blister-like lesions or larger, deeply-seated, painful masses that may be bruised or bleeding (Sivaseelan, 2021 ; Kudnig, 2022 ; Smith, 2003)

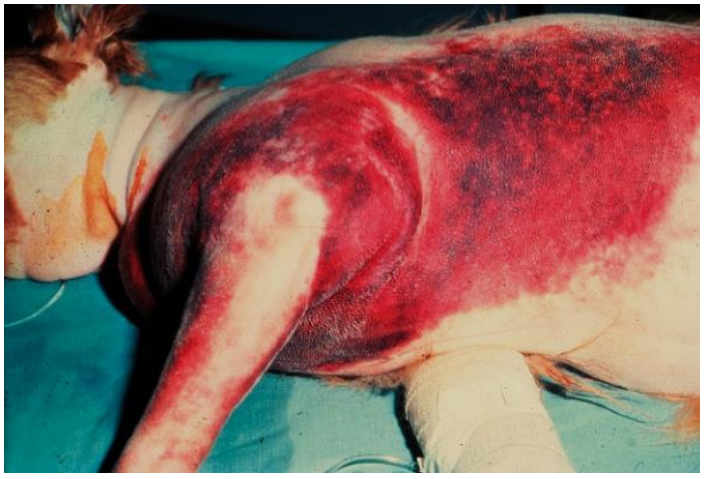

Figure 2. Clinical photograph of canine cutaneous hemangiosarcoma with extensive extravasation (Kiehl, 2016).

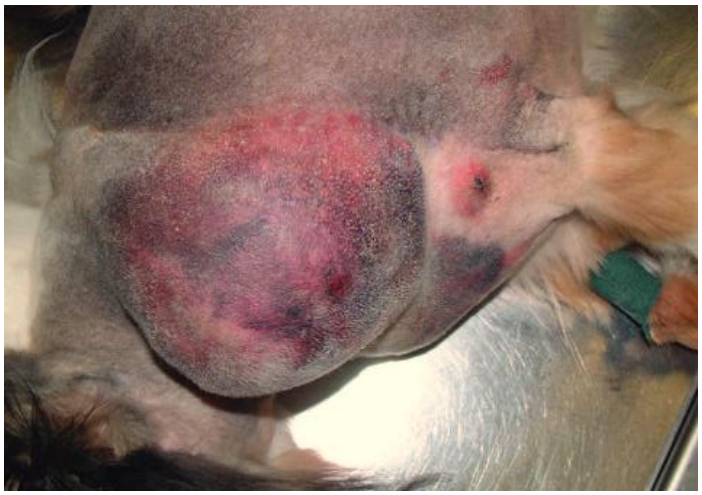

Figure 3. A dog with a subcutaneous hemangiosarcoma invading into the underlying musculature of the thoracic limb (stage III) with a typical bruised appearance (Vail, 2020).

5. Diagnosis

Complete staging for confirmed or suspected HSA typically includes comprehensive blood analysis (hematology, biochemistry, coagulation), thoracic radiographs, abdominal ultrasound, and potentially echocardiography. Anemia is common, either regenerative or nonregenerative, characterized by the presence of schistocytes, acanthocytes, and nucleated red blood cells. These cell abnormalities indicate microangiopathic damage, vasculitis, and acute hemorrhage. Neutrophilic leukocytosis, potentially due to a paraneoplastic syndrome or tumor necrosis, is also frequent. Thrombocytopenia is observed in 75-97% of cases, likely stemming from acute hemorrhage, intratumoral destruction, and coagulopathy. Nearly 50% of patients with visceral HSA will present with alterations in coagulation parameters (PT, PTT, FDP, fibrinogen, d-dimers), consistent with DIC. Serum biochemistry changes are often nonspecific but may

include hypoalbuminemia, azotemia, and elevated liver enzymes. Abdominal ultrasound is a crucial initial diagnostic tool as most patients exhibit primary visceral disease (Maruyama et al., 2005 ; Kobayashi et al., 2019 ; Witter et al., 2017).

Ultrasound typically reveals lesions with a heterogeneous appearance ranging from hypoechoic, targetoid, or mixed echogenicity with cavitation areas. Often, a peritoneal effusion accompanies these findings. Three-view thoracic radiographs are essential for routine metastatic disease screening in the lungs. HSA can manifest on these images in various ways, often described as a nodular pattern, sometimes with interstitial coalescing miliary features. Additionally, dogs with pericardial effusion from cardiac HSA may exhibit a globular cardiac silhouette on radiographs, with or without distension of the caudal vena cava. For confirmed cardiac HSA, echocardiography becomes the primary modality for identifying the primary tumor, with the presence of pericardial effusion aiding in better detection. While not routine, advanced imaging techniques like CT scans and MRI scans can be valuable in several scenarios: defining the anatomical origin and extent of the disease differentiating between benign and malignant lesions in the spleen and liver, and early detection of pulmonary metastases (Wisner, 2015 ; Penninck, 2015 ; Holloway, 2013).

Definitive diagnosis of HSA hinges on histopathological examination of tissue samples, typically obtained through splenectomy or necropsy. For solid tumors resembling other soft tissue sarcomas, IHC using vascular endothelial markers like CD31 /PECAM-1 and Factor VIII-related antigen (FVIII-R Ag) aids in confirming vascular origin. Both markers are recommended as some cancers may lack expression of either. To differentiate between hemangiosarcoma and lymphangiosarcoma, lymphatic vessel markers like LYVE-1 and PROX-1 may be employed. However, specific markers for distinguishing HSA cells from healthy ones are still unavailable. While fine-needle aspirate (FNA) cytology is attempted, its diagnostic yield for HSA is often low due to hemodilution. In rare instances where cytology is conclusive, it typically reveals large, pleomorphic spindle cells with multiple malignant features (Ferrer et al., 1995 ; Chu et al., 2023 ; Sabattini et al., 2009).

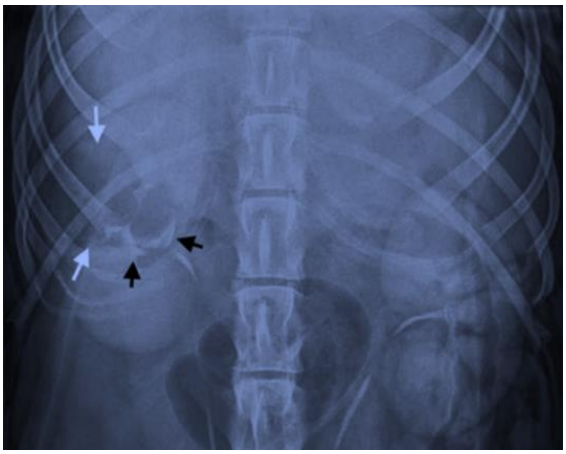

Figure 5. Renal haemangiosarcoma, the cranial and caudal poles have opacified normally, but an irregular lobulated filling defect (white arrows) occupies the central region of the right kidney, the pelvis is distorted and displaced (Holloway, 2013).

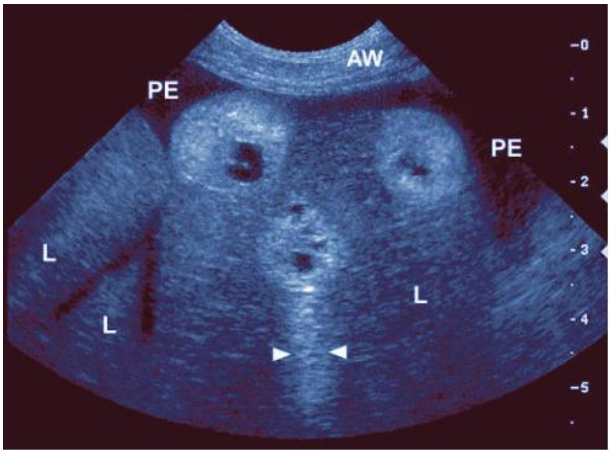

Figure 4. Hypoechoic to anechoic cavitary foci are found in some of these nodules and are associated with far enhancement (arrowheads), peritoneal effusion (PE) delineates the liver lobes (L); hepatic metastases were confirmed at surgery; AW, abdominal wall (Penninck, 2015).

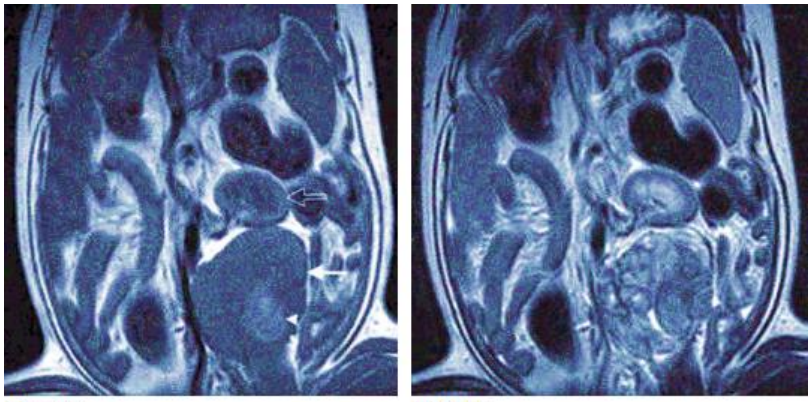

Figure 6. Retroperitoneal HSA, MRI, a large mass in the left retroperitoneal space (a: arrows) displacing the left kidney (a: open arrow). There is a focal, central T1 hyperintensity indicative of intratumoral hemorrhage (a: arrowhead). The internal portion of the mass is T1 hypointense (a) and heterogeneously T2 hyperintense (b) (Wisner, 2015).

6. Pathology

6.1. Gross morphology

Upon gross examination, splenic hemangiosarcomas typically present as

- Single or multiple, soft masses within the spleen, varying in size
- Dark red, mottled appearance with a potential "honeycomb" pattern due to tumor vasculature and bleeding

- Tan-white areas signifying solid tumor growth, requiring differentiation from necrosis, fibrosis, or fibrin deposits
- Potential dominance by a tumor-associated hematoma

Metastatic presence in the omentum, mesentery, liver, lungs, or brain is the most reliable gross indicator of splenic hemangiosarcoma. While serosal nodules from splenosis can mimic metastases, regional lymph node involvement is uncommon. Additionally, hemoabdomen occurs often, and omental adhesions form at splenic mass rupture sites, especially with hematoma (Maxie, 2016 ; Kudnig, 2022 ; Lashnits et al., 2020 ; Zachary, 2022).

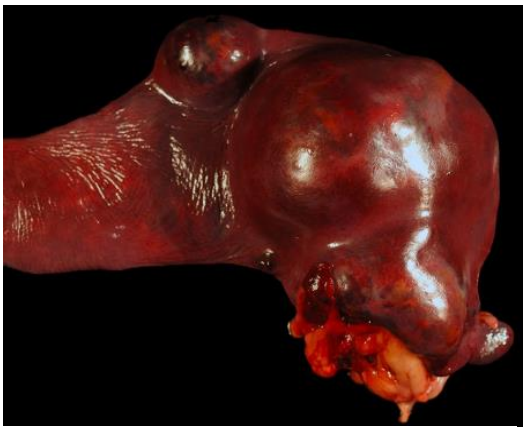

Figure 7. Primary hemangiosarcoma mass in a spleen in a dog (Lashnits, 2020).

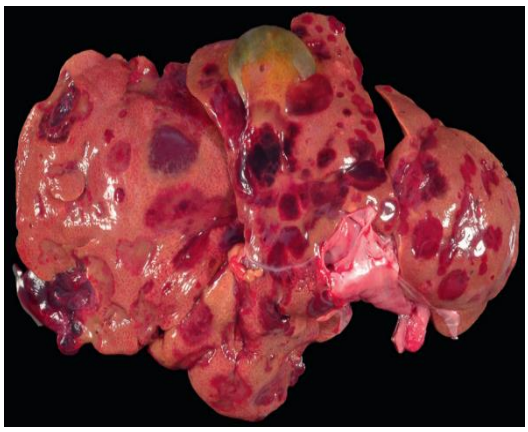

Figure 8. Hepatic hemangiosarcoma in the liver of a dog (Maxie, 2016).

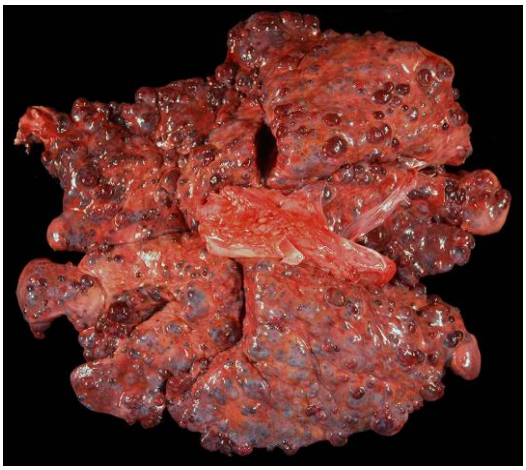

Figure 10. Metastatic hemangiosarcoma in the lungs with multifocal masses in all lobes (Lashnits, 2020).

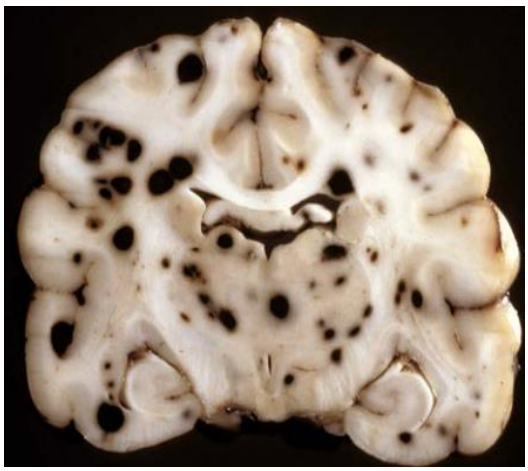

Figure 9. Hematogenous metastases, which appear as black nodules of various sizes,sometimes at the gray matter–white matter interface (Zachary, 2019).

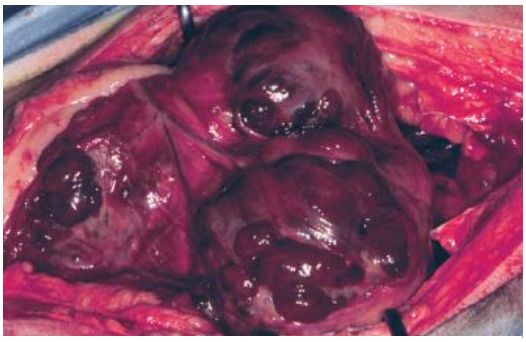

Figure 12. Intraoperative image of multifocal hemangiosarcoma within the spleen of a dog (Vail, 2020).

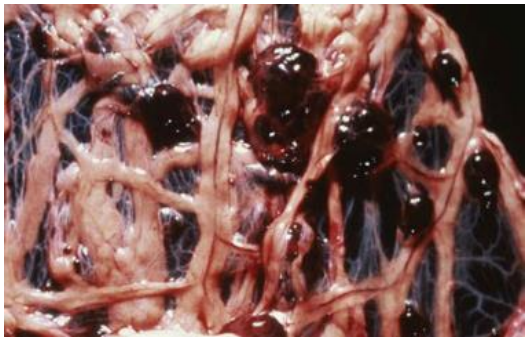

Figure 11. Splenic hemangiosarcoma with transcoelomic metastases (Zachary, 2019).

6.2. Cytological features

Because large areas of the tumor are filled with blood, one commonly only sees blood on aspirates of hemangiosarcomas, making the lesions difficult to distinguish from hematomas or hemangiomas. Evidence of previous hemorrhage (erythrophagocytosis and hemosiderin) is often present (Thrall, 2022). Tumor cells exhibit variable exfoliation patterns. They appear as pleomorphic spindle cells, observed either individually or aggregated/sheet-like, potentially forming cords. These spindle cells are generally plump with a moderate amount of medium-blue cytoplasm tapering at their ends, sometimes with long extensions. Although erythrophagia by neoplastic cells has been reported, it is not a specific feature for diagnosing HSA. The nuclei are ovoid-shaped with finely granular chromatin and multiple prominent basophilic nucleoli. Multinucleation and mitotic activity are frequently observed. Additionally, significant anisocytosis and anisokaryosis are characteristic findings.As mentioned before, these tumors are difficult to differentiate definitively from other sarcomas cytologically (Weiss, 2010 ; Burton, 2024).

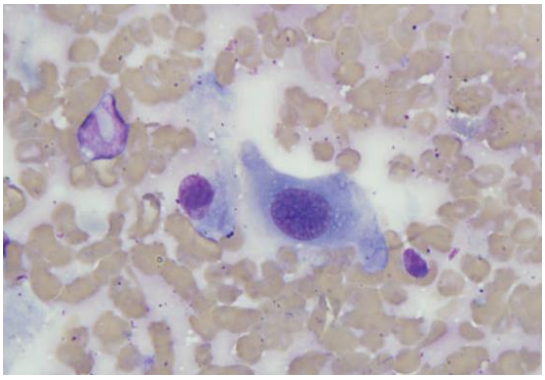

Figure 13. Penile HSA. Individualized large spindle-shaped cells with abundant basophilic cytoplasm, often several cytoplasmic vacuoles, and a large ovoid nucleus are seen (1,000× magnification) (Barger, 2017).

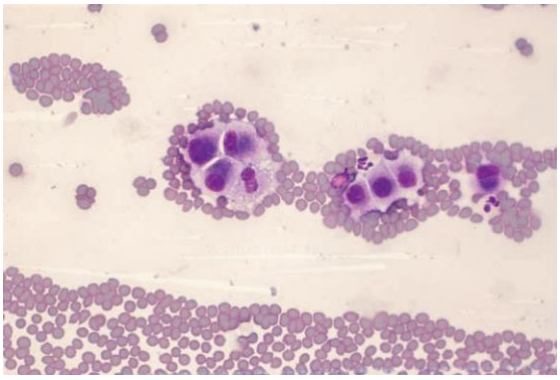

Figure 14. Pericardial effusion from a dog with hemangiosarcoma. Low numbers of pleomorphic plump mesenchymal cells are noted (400× magnification) (Barger, 2017).

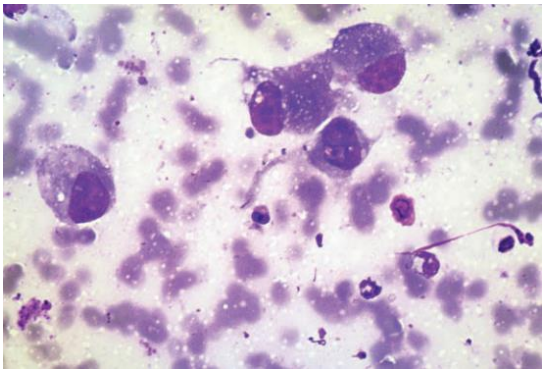

Figure 16. FNA, splenic hemangiosarcoma. Large globoid, to stellate hemangioblasts, with deeply basophilic cytoplasm, and several microvacuoles (600× magnification) (Barger, 2017).

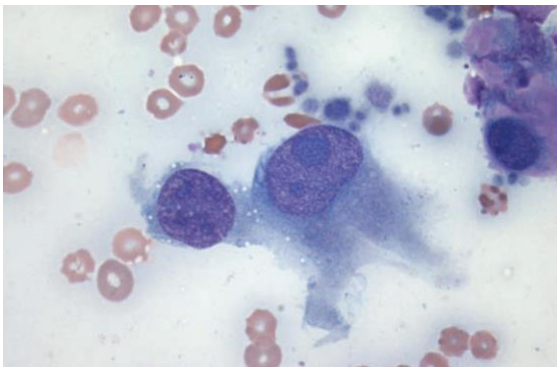

Figure 15. Subcutaneous HSA. Mesenchymal cells. Anisocytosis, anisokaryosis, atypical nuclear shape, stippled chromatin, multiple prominent nucleoli, and variably-sized nucleoli are shown (1,000× magnification) (Barger, 2017).

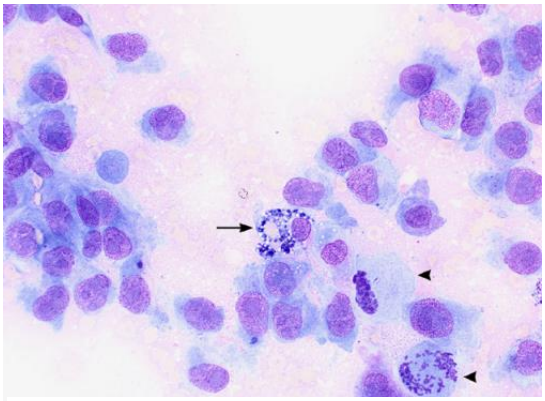

Figure 18. Hemangiosarcoma (cutaneous), dog. Note the increased mitotic figures (arrowheads) and erythrophagia/ hemosiderin pigment within neoplastic cells (arrow) (500× magnification) (Burton, 2024).

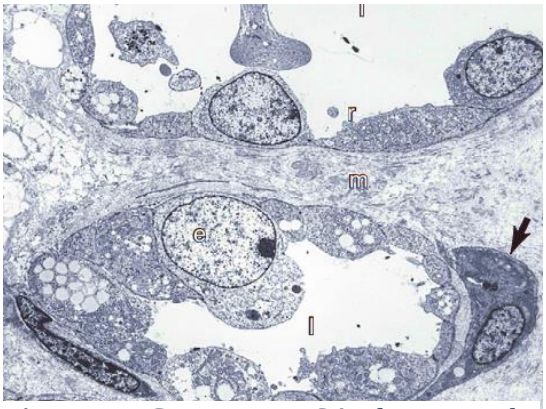

Figure 17. Cutaneous HSA, dog. Note the increased mitotic figures (arrowheads) and erythrophagia/ hemosiderin pigment within neoplastic cells (arrow) (500× magnification) (Raskin, 2010).

6.3. Histological features

Hemangiosarcoma diagnosis relies on the identification of neoplastic endothelial cells forming blood vascular spaces. While this presentation can be readily apparent in some cases, others may require comprehensive histological section evaluation to confirm or rule out the diagnosis. Non-encapsulated, invasive neoplasms typically exhibit variably sized, irregularly shaped blood vascular spaces separated by thin septae or more solid areas. These spaces are often traversed by slender trabeculae and contain blood. The growth patterns are diverse, and the presence of capillary, cavernous, and solid areas is frequently observed. Epithelioid areas, characterized by polygonal cells with round nuclei and abundant eosinophilic cytoplasm, can occasionally be found. Neoplastic endothelial cells lining these spaces typically exhibit hyperchromasia, plumpness, a spindled morphology, and are larger than normal endothelial cells. Additionally, cell size variations (anisocytosis), nuclear size variations (anisokaryosis), and mitotic figures are often present, although the extent can vary. The presence of mitotic figures in neoplastic endothelial cells, particularly within the spleen, provides strong support for a hemangiosarcoma diagnosis. It's crucial to differentiate these from occasional mitotic figures observed in neovascularization associated with granulation tissue or other reparative processes. Multinucleated cells may be present but are uncommon. Collagenous trabeculae spanning vascular spaces are a crucial feature unique to proliferative lesions of endothelial origin and should be actively sought during examination. Hemangiosarcomas typically exhibit growth without recruitment of normal blood vessels, although resident vessels can be entrapped. Patchy to large areas of necrosis, hemorrhage, and/or fibrin deposition are also frequently observed. The diverse cellular composition of aged canine red pulp, often with inflammatory cells, can make histological assessment of lesions complex. Differentiating solid-growth hemangiosarcoma from stromal spleen sarcomas presents particular difficulties. Large, hyperchromatic spindle cells arranged in linear arrays without specific patterning and scant connective tissue matrix typify this challenge. While numerous individual red cells percolating between closely apposed neoplastic cells offer a strong clue for solid hemangiosarcoma diagnosis, this feature is inconsistent and can be mistaken for hemorrhage. Littoral cell angiosarcoma, while rare, has been observed in canines with spleen involvement and metastasis. Histological hallmarks include anastomosing microvascular channels and multifocal microvascular papillary fronds. Neoplastic cells likely originate from sinusoidal red pulp lining cells, exhibiting both endothelial and histiocytic markers. These cells may also demonstrate erythrophagocytosis. Epithelioid hemangiosarcoma, found in multiple species, usually manifests in canine skin but can present viscerally with splenic involvement. Epithelioid

morphology is dominant, though small areas may retain classical endothelial morphology and vascular channel formation. Retiform hemangioendothelioma, also seen in dogs, shows widespread tissue involvement including the spleen. It features distinctive elongate, arborizing vascular channels lined by neoplastic endothelial cells with prominent nuclei. It's crucial to note that while non-neoplastic sinusoidal telangiectasis and splenic injury can mimic the appearance of hemangiosarcoma, they generally lack neoplastic cellular morphology and show evidence of parenchymal damage. IHC isn't typically required for diagnosing most hemangiosarcoma cases, it can offer valuable assistance in identifying: solid hemangiosarcomas (difficult to distinguish from other types of sarcomas due to their solid growth pattern) and poorly differentiated neoplasms (they lack the clear morphological features of well-differentiated hemangiosarcomas) (Zachary, 2022 ; Vail, 2020 ; Harvey , 2012 ; Chu et al., 2023, Bertazzolo et al., 2005).

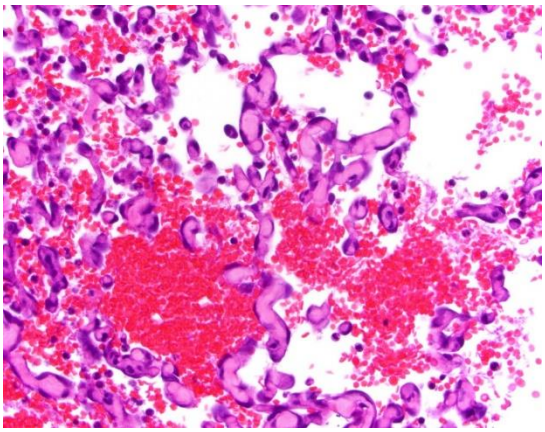

Figure 19. Hemangiosarcoma tumor section shows collagen bundles surrounded by neoplastic endothelial cells and appear to form “inside out” blood vessels (Mukai et al., 2020).

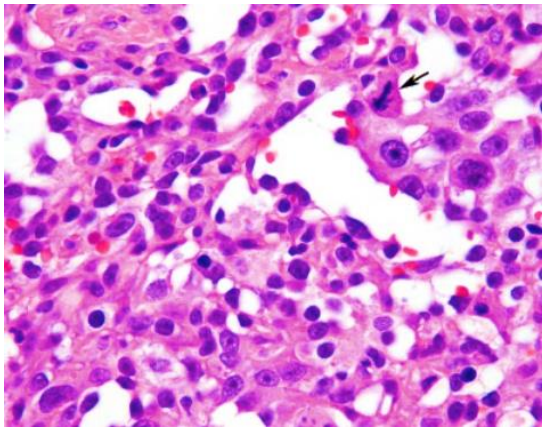

Figure 20. Hemangiosarcoma of bone, FNA, variation in nuclear size, prominent nucleoli, and the atypical mitotic figure (arrow) seen cytologically. Note the vascular channels have no visible walls and are rimmed only by neoplastic cells (Meuten, 2017).

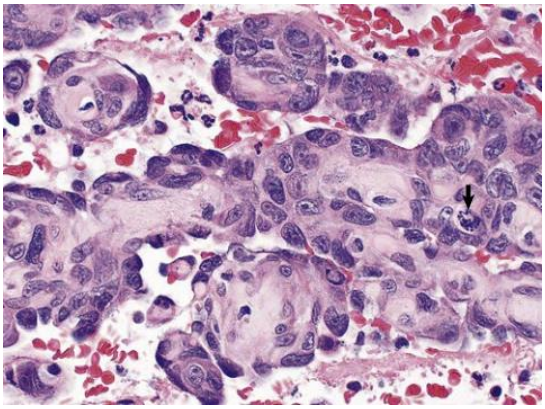

Figure 21. Splenic hemangiosarcoma, dog, neoplastic endothelial cells form haphazardly organized blood-filled vascular channels, mitotic figure (arrow) (Zachary, 2019).

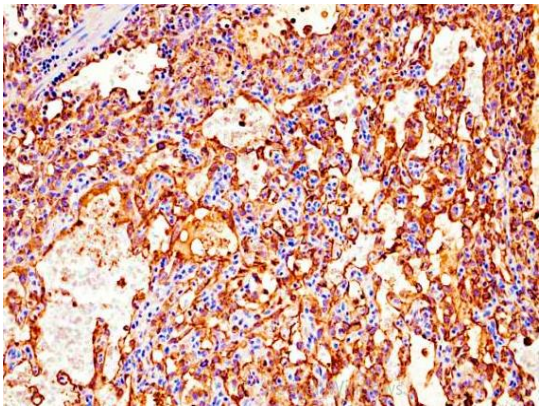

Figure 22. Neoplastic endothelial cells show diffuse dark brown cytoplasmic and membranous staining: CD31 (Kodama et al., 2009).

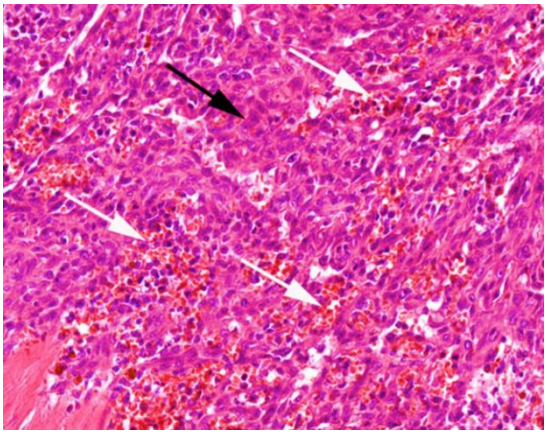

Figure 23. Few erythrocytes and vessel-like cavities (white arrows) are interspersed between abundant mostly plump neoplastic endothelial cells (black arrows) (Klopfleisch, 2016).

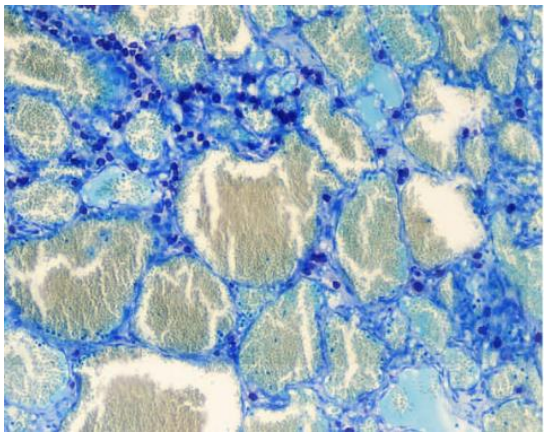

Figure 24. Cutaneous haemangiomas showing interstitial infiltration of mast cells (Sabattini and Bettiniet, 2009).

6.4. Hematology

Canine hemangiosarcoma presents with a constellation of hematological abnormalities. The most frequently reported findings include regenerative anemia with poikilocytes and rubricytes, neutrophilia with a left shift, and thrombocytopenia.

6.4.1. Erythrocytes

Anemia is usually mild to moderate but can be severe. Kleine et al., (1970) found anemia in 18 of 29 dogs with HSA. The packed cell volume (PCV) was 0.06-0.32 UL with a mean of 0.24 yL. Hemoglobin values were 60-113 g/L with a mean of 78 g/L (reference Iimits, 120- 180 g/L). The anemia is described as very responsive with prominent polychromasia, reticuiocytosis

and anisocytosis. However, the anemia may also be non-responsive as is reported with primary cardiac and prostatic HSA (Tant, 1998).

Poikilocytosis is a general term for abnormally shaped red blood cells, often seen in hemangiosarcoma but not specific to this disease. In dogs, hemangiosarcoma is more commonly associated with acanthocytosis (irregularly spaced spiny projections). Nucleated Red Blood Cells (nRBCs): Low numbers of these immature red blood cells might be observed in splenectomized dogs with hemangiosarcoma (spleen plays a role in RBC maturation). Keratocytes are rare, spiny red blood cells seen in some cases of chronic doxorubicin administration (a chemotherapy drug) and hemangiosarcoma. Echinocytes are red blood cells with evenly spaced, small protrusions on the surface, they are more frequent in dogs with various cancers, including hemangiosarcoma. Acanthocytes are red blood cells with irregularly spaced, spiny projections. They are associated with increased cholesterol in the red blood cell membrane and can be seen in hemangiosarcoma and other conditions causing RBC fragmentation. Schistocytes are fragmented red blood cells with pointed ends, often seen in hemangiosarcoma and other conditions like iron deficiency anemia (Thrall, 2022 ; Weiss, 2010 ; Harvey, 2023 ; Burton, 2024)
Immune-Mediated Hemolytic Anemia (IMHA): hemangiosarcoma can be associated with IMHA, where the immune system attacks red blood cells. The exact mechanisms are unclear.

6.4.2. Leukocytes

Neutrophilia is not a consistent finding in canine hemangiosarcoma. Shiu et al. (2011) reported neutrophilia in only 26% of their study population. This suggests that neutrophilia might be a conditionally associated feature, manifesting in specific circumstances. Neutrophilic leukocytosis may occur in cases of cutaneous HSA resulting from the presence of inflammation and tumor necrosis and is more commonly observed in dogs with larger and infiltrative tumors (Tant, 1998).

6.4.3. Platelets

Canine hemangiosarcoma can disrupt platelet function in several ways. Increased platelet consumption is a common feature, occurring due to two main mechanisms:

- Disseminated Intravascular Coagulation (DIC) and Thromboembolism: In some cases, hemangiosarcoma can trigger DIC, a condition where abnormal blood clotting occurs throughout the body. This widespread clotting consumes platelets. Additionally, tumor-related blood clots (thromboembolism) can also lead to platelet depletion.

- Tumor-induced Hemorrhage: Hemangiosarcomas are prone to bleeding, which directly reduces platelet numbers.

However, a significantly decreased platelet count (thrombocytopenia) is not a universal finding unless there's a severe and acute hemorrhage from a ruptured tumor. Thrombocytopenia can occur with various cancers in dogs, but it's most frequently observed in lymphomas and hemangiosarcomas. In some hemangiosarcoma cases, extensive clotting might be confined to the tumor and surrounding blood vessels (local intravascular coagulation). This scenario can present laboratory findings similar to DIC, making it challenging to differentiate between the two conditions (Burton, 2024 ; Harvey, 2023 ; Weiss, 2010)

6.4.4. Blood chemistry

Hypofibrinogenemia has been reported in some hemangiosarcoma cases, potentially linked to localized coagulation or DIC. The exact immunologic mechanism remains unclear (Meuten, 2017).

Coagulation Parameter	% Abnormal
Prolonged PT	12.5
Prolonged APTT	46
Thrombocytopenia	75-97
Increased fibrin degradation products	46-93
Hypofibrinogenemia	8-46
Criteria for DIC	47-50

Table 6. Coagulation abnormalities in dogs with hemangiosarcoma (Meuten, 2017).
Abbreviations: PT, prothrombin time; APTT, activated partial thromboplastin time; DIC, disseminated intravascular coagulation.

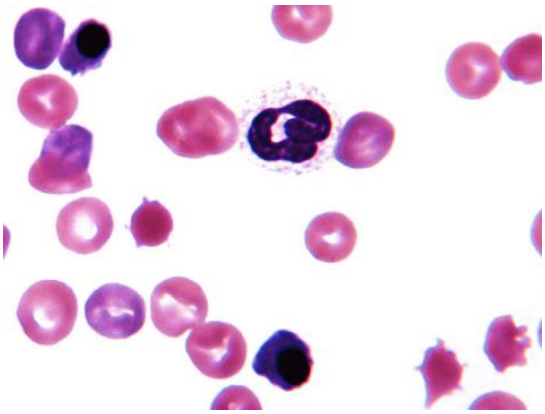

Figure 26. Cutaneous hemangiomas showing interstitial infiltration of mast cells (Harvey, 2012).

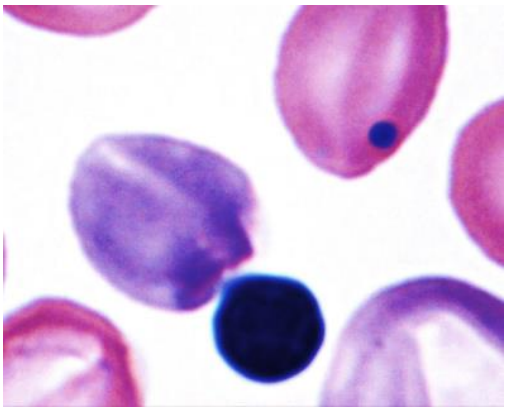

Figure 25. Nuclear extrusion of metarubricytes to form canine reticulocytes, blood film from a dog with a hemolytic anemia secondary to hemangiosarcoma (Harvey, 2012).

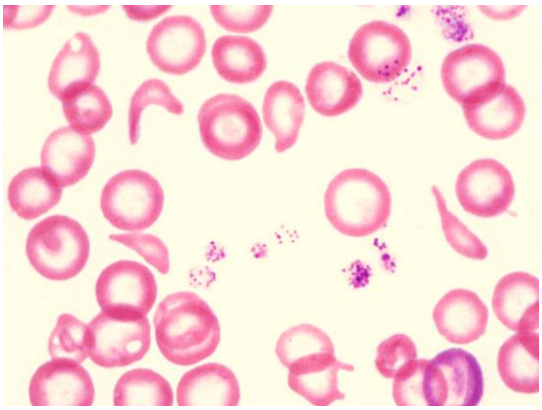

Figure 28. Hypochromic erythrocytes in a canine blood, a microcytic hypochromic anemia with poikilocytosis (including keratocytes, schistocytes, and dacryocytes) was present (Harvey, 2012).

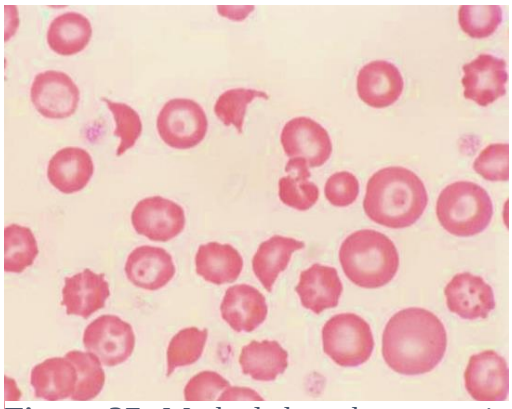

Figure 27. Marked thrombocytopenia and presence of several schistocytes or fragmented RBCs are indications that thrombosis or DIC is occurring (100x magnification) (Harvey, 2012).

6.5. Urinalysis

Hemangiosarcoma and transitional cell carcinoma of the renal pelvis frequently manifest with hematuria, while proteinuria is a more common yet less specific finding in urinary analysis. While cytological examination of urinary sediment can occasionally identify tumor cells; however, this finding has limited diagnostic reliability (Rizzi, 2017 ; Wang and Su, 2001).

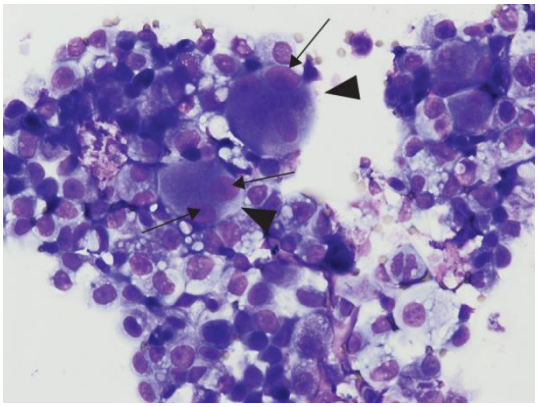

Figure 29. Urine sample, neoplastic cells with variable nuclear sizes, multinucleated cells (arrow heads), prominent nucleoli (arrows) (500x magnification) (Rizzi, 2017).

Parameter	Ref. value	Days			
		1	9	20	26
Glucose	–	–	–	–	–
Bilirubin	±	+	++	–	–
Ketone	–	–	–	–	–
Specific gravity	1.015 – 1.045	> 1.040	> 1.040	1.035	1.018
Occult blood	–	> +++	++++	> +++	> ++++
pH	6 – 8	6.5	6.5 – 7.0	6.0	6.0
Protein	–	++	++++	+++	+++
Urobilinogen	0.1 – 1	0.1	0.1	0.1	0.1
Nitrate	–	–	–	–	–
Sediments					
RBC/HPF	< 4 – 5	Numerous	Numerous	Numerous	Numerous
WBC/HPF	< 5 – 8	5 – 15	5 – 15	12 – 20	Numerous
Squamous epithelium/HPF		1 – 4	0 – 5	0 – 4	5 – 10
Cast	–	–	–	–	Struvite
Crystal	–	–	–	–	–
Microorganism	–	–	–	–	–

Table 7. Urinalysis of a male dog presented with stranguria and hematuria, it shows high urine specific gravity (> 1.040 g/mL), hematuria, proteinuria and mild bilirubinuria. Necropsy revealed that the HSA in the kidneys was the major cause of hematuria (Wang, 2001).
Abbreviations: RBC, red blood cell; WBC, white blood cell; HPF, high-power field.

6.6. Biomarkers

6.6.1. Serum markers

Serum biomarkers hold promise for non-invasive screening or diagnosis of canine HSA due to the ease of sample collection. However, a significant challenge lies in ensuring biomarker specificity. Several previously reported markers, including elevated α-1 acid glycoprotein (AGP), thrombin-antithrombin (TAT) complex, and thymidine kinase 1 (TK-1), have limitations. These markers exhibit increased concentrations not only in HSA but also in various malignant and benign tumors. Similarly, while serum VEGF and ferritin show elevation in HSA compared to healthy dogs, they cannot distinguish HSA from HSA-mimicking splenic lesions like hematomas. Recent research has identified potential biomarkers with improved specificity for HSA diagnosis. Serum collagen XXVII alpha-1 concentration demonstrates promise, exhibiting higher levels in HSA patients compared to healthy dogs or those with other cancers and inflammatory diseases. Additionally, serum markers like Big endothelin-1 (ET-1), circulating microRNAs (miR-214 and miR-126), and specific glycoproteins show potential for differentiating HSA from benign splenic lesions (Fukumoto et al., 2015 ; Kirby et al., 2011 ; Heishima et al., 2015 ; Yuki et al., 2011).

Plasma nucleosome concentrations may have potential as a non-invasive detection valuable diagnostic tool. Studies by Wilson-Robles et al. (2021) demonstrate their consistency in healthy dogs regardless of age, gender, or body size. Notably, elevated nucleosome levels are observed even in early-stage HSA, exceeding 80% of evaluated patients.

6.6.2. Tissue markers

Exploring canine hemangiosarcoma through tissue markers serves three key purposes: therapeutic target identification, prognostic development, and studying pathogenesis. Factor VIII-related antigen (vWF) was initially identified as a marker for endothelial cells in both canine HSA and HA, exhibiting positive staining in 100% of HA samples but only 89% of HSA samples. CD44, a cell surface adhesion molecule, was not expressed in any HAs but was immunoreactive in over half of HSAs. Vimentin, an intermediate filament protein, was expressed in both neoplastic and non-neoplastic endothelial cells (ECs). This finding suggests vimentin IHC could be valuable in diagnosing atypical HSA variants, such as epithelioid HSA. PECAM (CD31) is reliable marker for canine endothelial cells. Immunohistochemistry revealed its superior performance in detecting HSA (100% positive samples) compared to vWF (73% positive samples). Consequently, CD31, along with vWF, has become widely used in immunohistochemical protocols to differentiate vascular tumors from other soft tissue tumors. Ulex europaeus agglutinin-1 (UEA-1) lectin, while not detected on healthy canine endothelial cells, showed variable staining on HA and HSA tissues, highlighting its limitations in providing consistent results (Ferrer et al., 1995 ; Chu et al., 2023 ; Sabattini et Bettiniet, 2009)

Immunohistochemical analysis reveals variations in the expression levels of proteins like Claudin-5, VEGF, flk-1, VEGFR3, CD117, AGP-α, and PDGF-β between malignant HSA and benign HA tissues. These findings point towards the potential involvement of these proteins in the malignant behavior of HAS (Oungsakul, 2020).

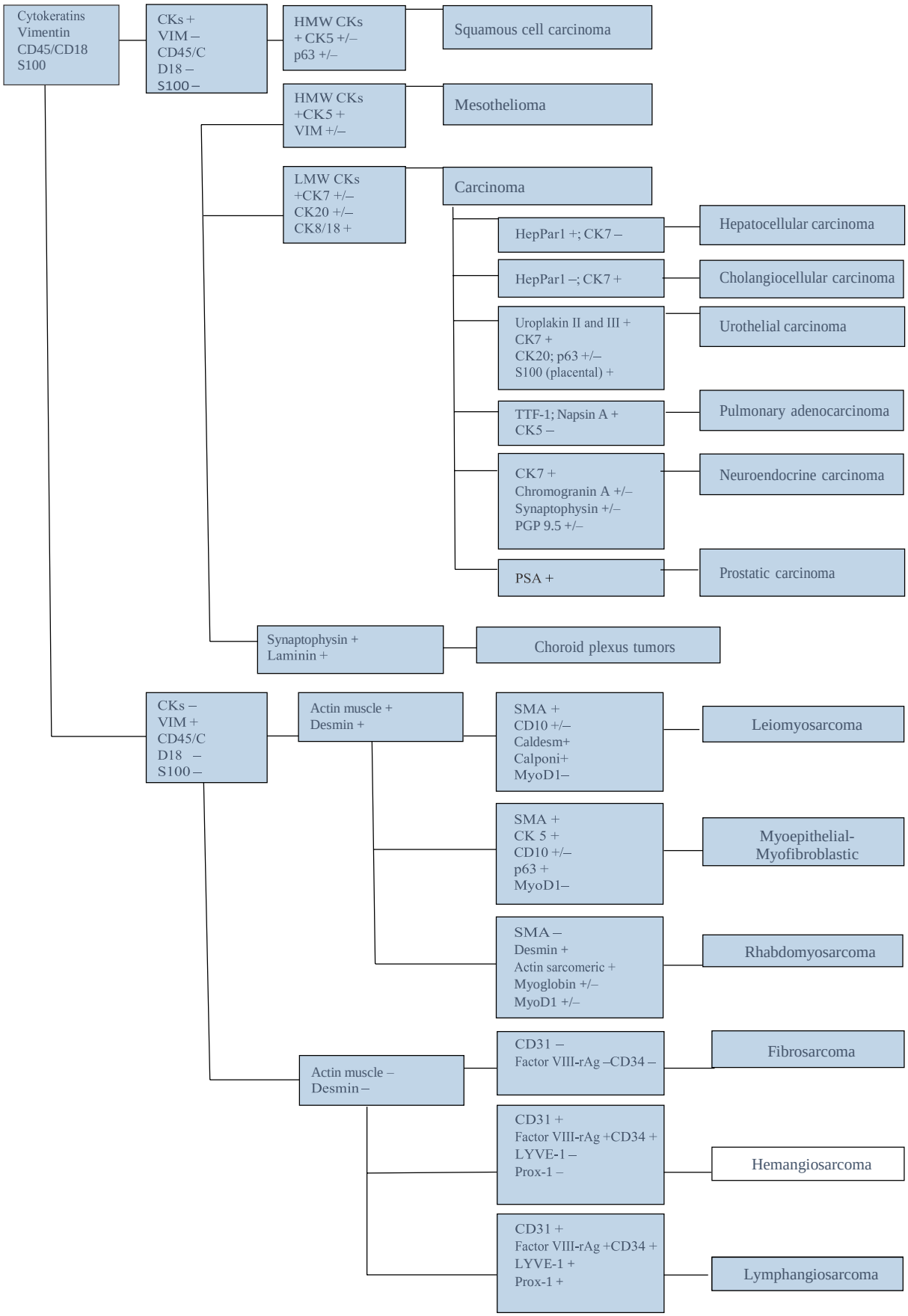

Figure 30. Algorithm to HSA diagnosis using immunohistochemistry (Meuten, 2017).

7. Treatment

7.1. Surgery

Surgical removal remains the go-to method of treatment for almost all dogs with localized, non-metastatic HSA, the specific surgical approach varies depending on the location of the primary tumor.

Type	Methode of choice
Visceral hemangiosarcoma	
Spleen	Splenectomy is the primary treatment, Careful abdominal exploration is crucial to identify and remove any suspicious lesions in the liver or omentum during surgery
Liver (solitary)	Liver lobectomy is the recommended approach
Kidney (solitary)	Nephrectomy is indicated
Heart	Surgery is rarely performed for primary cardiac tumors. However, right atrial appendage masses might be resected via thoracotomy or thoracoscopy
Cutaneous hemangiosarcoma	Surgical considerations are similar to those for other malignant skin tumors and soft tissue sarcomas.
Dermis	Typically discrete, requiring margins of 1-2 cm with a fascial plane depth
Subcutaneous and intramuscular tissue	Often locally invasive, with edema and bleeding. Wider margins may be difficult, and amputation might be necessary for limb tumors.

Table 8. Surgical oncology treatment of hemangiosarcoma (Sivaseelan, 2021 ; Kudnig, 2022).

7.2. Chemotherapy

Due to the high risk of metastasis in canine hemangiosarcoma, adjuvant chemotherapy is recommended for almost all cases except for most purely dermal tumors. Doxorubicin (DOX) is considered the most effective single agent against HSA, used either alone or in combination with other drugs like cyclophosphamide (CYC) to form protocols like AC (DOX and CYC). Other potentially active single agents include ifosfamide, liposomal doxorubicin (Doxil, Caelyx), and epirubicin. Given the vascular nature of HSA, targeting angiogenesis (blood vessel formation) offers a promising therapeutic approach. Metronomic chemotherapy, a low-dose approach with or without additional drugs, is gaining interest for its potential anti-angiogenic properties in HSA treatment (Crump, 2011 ; Dobson, 2016).

7.3. Immunotherapy

Research on biologic therapies for HSA remains scarce. An early investigation using a combined killed bacterial vaccine and DOX chemotherapy after splenectomy for HSA showed some improvement in survival time compared to surgery alone. Another study explored

combining adjuvant AC chemotherapy with L-MTP-PE, an immunomodulator that boosts monocyte activity against tumors. This combination led to a significantly longer median survival time (MST) compared to using AC alone (Vail et al., 1995).

7.4. Radiation Therapy

RT is rarely employed for HSA due to predominantly visceral location and high metastatic rate (Morris, 2001).

7.5. Targeted Therapies

While conventional therapies offer limited improvement in survival for dogs with HSA, the potential for more precise treatment options remains Intriguing. Research has identified the expression of receptor tyrosine kinase (RTK) family members in canine HSA, including PDGF receptor (PDGFR), VEGF receptor (VEGFR), and stem cell factor receptor (KIT). These RTKs play a role in tumor growth and survival. Targeted therapy with small molecule inhibitors like Masitinib (PDGF and KIT inhibitor), Imatinib (KIT and PDGFR inhibitor), and Dasatinib (KIT, PDGFR, and SRC inhibitor) shows promise in the lab. However, achieving effective drug concentrations without significant side effects in living animals can be challenging. A new approach, eBAT (a bispecific inhibitor), has shown encouraging results in a small study. Dogs receiving eBAT before standard DOX chemotherapy had a significantly longer median survival time (8.5 months) and a higher 6-month survival rate (70.6%) compared to those receiving DOX alone (Borgatti et al., 2017).

8. Prognosis

Canine visceral HSA carries a bleak prognosis. The median survival time for dogs diagnosed with this aggressive cancer varies widely (19 to 240 days) depending on the disease stage at diagnosis. While adjunctive therapies like chemotherapy may offer some improvement in survival, the statistics are sobering. Less than 10% of dogs survive beyond one year, with about 30% surviving for up to two months. This stands in stark contrast to the significantly better prognosis for cutaneous hemangiosarcoma, where the median survival time can reach up to 987 days. Although some studies suggest that well-differentiated tumors may correlate with longer disease-free intervals (DFI) or survival time after splenectomy (Göritz et al., 2013 ; Vail et al., 1995; Shiu et al., 2011).

Treatment	Dogs (n°)	Median Survival Time (days)
Splenectomy	Various	19-86
Splenectomy + MBV	10	91
Splenectomy + MBV + VMC	10	117
Splenectomy + LDCC	9	178
Splenectomy + Ifosfamide	6	142
Splenectomy + Ifosfamide/DOX	13	123
Splenectomy + Epirubicin	59	144
Splenectomy + Doxil	14	132
Splenectomy + VAC	6	145
Splenectomy + AC	Various	141-179
Splenectomy + AC + L-MTP-PE	16	273

Table 9. Comparison of survival times in dogs with splenic hemangiosarcoma (Meuten, 2017). Abbreviations: MBV, Mixed bacterial vaccine; VMC, vincristine, methotrexate, cyclophosphamide; LDCC, Low-dose, continuous chemotherapy; DOX, doxorubicin; VAC, vincristine, doxorubicin, cyclophosphamide; AC, doxorubicin, cyclophosphamide; L-MTP-PE, liposome muramyl tripeptide phosphatidylethanolamine.

Chapter IV Etiopathogenesis of Canine Hemangiosarcoma

1. Etiology

The exact cause of hemangiosarcoma in dogs remains unknown. While experimental studies have induced visceral tumors in beagles using radiation exposure (both external and inhaled), and sunlight exposure has been suggested as a risk factor for cutaneous HSA. Dogs that were exposed to aerosolized radionuclides developed primary pulmonary, bone, and liver HSAs at a high rate and usually died of their tumor. Ongoing research explores potential connections between vector-borne pathogens (VBPs) such as Babesia spp. and Bartonella and various canine splenic diseases, including HSA. These investigations may provide valuable insights into the potential role of VBPs in canine splenic pathology. A definitive link between any specific agent and naturally occurring HSA in dogs has yet to be established (Tant, 1995).

1.1. Hypoxia and inflammation

Laifenfeld et al. (2009) propose that hypoxia and inflammation play key roles in hepatic angiosarcoma development. Interestingly, the effects extend beyond the liver itself, impacting the bone marrow and spleen. Their study used 2-Butoxyethanol (2-BE) to induce red blood cell hemolysis, creating a hypoxic environment. This hypoxia:

- Triggered liver inflammation: inflammation stimulated endothelial cell (EC) proliferation within the liver.
- Activated Erythropoietin signaling: that promotes the differentiation of endothelial cell progenitor cells (EPCs) and further endothelial cell proliferation in the bone marrow and spleen. These newly formed cells were then recruited to the tumor site.

During the phase of endothelial cell proliferation and EPC/EC recruitment, mechanisms that maintain genomic stability likely failed at some point. This failure allowed the mutated, proliferating cells to develop into hemangiosarcoma.

1.2. Radionuclides

Hemangiosarcomas have been observed in beagles with internally deposited radionuclides. In experiments where beagles were exposed to inhaled radionuclides, such as ^{144}Ce and ^{90}Sr, hemangiosarcomas were found to be the major cause of death in dogs dying within 2 years after exposure. The incidence of hemangiosarcomas was over 40% among the bone-related sarcomas seen in dogs exposed to ^{144}Ce or ^{90}Sr. In dogs exposed to $^{144}CeCl_3$, a relatively soluble form of the β-emitting radionuclide, liver tumors, including hepatic hemangiosarcomas, were the most

frequently observed neoplasms . In beagles exposed to graded activity levels of $^{91}YCl_3$, $^{144}CeCl_3$, or $^{90}SrCl_2$, squamous cell carcinomas associated with the nasal cavity, including hemangiosarcoma, were observed . The occurrence of hemangiosarcomas in beagles with internally deposited radionuclides suggests a potential risk of these tumors in humans exposed to similar radionuclides (Benjamin et al., 1975 ; Hahn et al., 1996 ; Raabe, 2010 ; Taylor et al., 1991)

1.3. Radiation exposure

Exposure to sunlight is considered a significant risk factor to develop cutaneous hemangiosarcomas, Hargis et al. (1992)'s findings suggest an association between solar radiation and the development of hemangiomas and hemangiosarcomas in dogs (approximately 18% of dogs with dermal but not subcutaneous hemangiomas and hemangiosarcomas had solar elastosis in skin adjacent to the tumor). They found that dogs with short hair coats and lightly pigmented skin had a higher incidence of these tumors. Specifically, the authors note that the distribution of these tumors in dogs with lightly pigmented skin and short hair coats is similar to the distribution of sun-induced tumors in humans. Additionally, the predilection for these tumors to occur in the ventral glabrous skins (63% dogs with hemangiomas, 69% dogs with hemangiosarcomas), which is an area that is less protected from the sun, further supports the hypothesis.

The study concludes that solar radiation exposure likely plays a role in the development of dermal and subcutaneous hemangiomas and hemangiosarcomas in dogs.

1.4. Infectious agents

No definitive infectious cause has been established for HSA. While some studies have suggested potential links. One study reported an association between HSA and Leishmaniasis in three dogs (Margarito et al., 1994). Another found a statistically higher prevalence of Bartonella spp. DNA in HSA tumors compared to control spleens from healthy dogs. Interestingly, this study also detected Bartonella DNA in healthy tissues from the same dogs, not just the tumoral tissue (Lashnits et al., 2022 ; Movilla et al., 2017). However, these findings alone do not conclusively demonstrate an infectious origin for HSA. Further research is needed to explore these potential associations.

2. Pathogenesis

2.1. Ontogeny of canine HSA

Canine HSA is a malignant neoplasm arising from vascular endothelial cells. However, its precise cellular origin still remains to be elucidated. Two primary hypotheses have been proposed to explain the developmental origin of HSA (Dickerson et al., 2005).

Hypothesis 1: Transformation of Mature Endothelial Cells, his hypothesis posits that HSA originates from differentiated endothelial cells within the lining of blood vessels. These cells, having matured beyond the angioblast stage, acquire mutations that confer malignant potential. This model presumes that any endothelial cell with proliferative capacity can undergo transformation and retain the ability for self-renewal. Tumor development, under this perspective, would be a stochastic process driven by the selection of advantageous mutations promoting proliferation and survival (Dickerson et al., 2005).

Hypothesis 2: Hemangiosarcoma from Hemangioblasts, the competing hypothesis proposes a distinct origin for HSA. It suggests that the tumor derives from incompletely differentiated, multipotential stem cells derived from the bone marrow. These precursor cells, termed hemangioblasts, are near or at the stage of commitment towards the endothelial lineage (Schatteman et Awad, 2004)

Lamerato-Kozicki et al. (2006) employed flow cytometry and immunofluorescence microscopy to investigate the cellular origin of HSA. Their study focused on six HSA cell lines, analyzing the expression of specific surface proteins.

- Markers of bone marrow precursors: The researchers examined proteins restricted to bone marrow precursor cells, such as c-kit, CD34, and $CD133^{+}$.
- Markers of lineage commitment: They also evaluated proteins that define lineage committed cells (CD3, CD21, CD11b, CD105, CD146, $\alpha_v\beta_3$-integrin).
- Hemangioblastic markers: Additionally, CD14 and CD45 were assessed, as these markers are expressed by hemangioblastic cells at various stages of differentiation.

The data suggest that HSA might originate from bone marrow precursors that become arrested in their development at the hemangioblast to angioblast stage, these precursor cells were found to express c-kit, CD34, $CD133^{+}$, and sometimes CD45. However, the current sample size might not be sufficient to determine if HSAs arising in different locations exhibit consistent differences in protein expression patterns. An alternative interpretation is that the tumors might

be expressing these lineage-associated proteins abnormally, not necessarily reflecting a true arrest in differentiation. The authors argue against this possibility and believe it's improbable that each tumor would independently reactivate such specific expression patterns without a pre-existing genetic code linked to their bone marrow origin.

The possibility that circulating HSA-like cells originate from normal bone marrow was also explored, however, while chronic bleeding or tumor-derived cytokines could potentially mobilize normal bone marrow-derived EPCs, the data suggests that anemia significantly contributing to the release of EPCs in dogs with HSA is unlikely. While the possibility that tumor-derived VEGF and other cytokines mobilize EPCs can't be entirely ruled out, two key observations suggest this is unlikely.

Firstly, VEGF levels appear similar in both HSA and splenic hematomas, indicating it might not be the sole mobilizing factor in HSA. Secondly, the circulating EPCs displayed distinct and varied protein expression patterns between dogs with HSA. Mobilized normal EPCs from bone marrow, in contrast, would be expected to exhibit a more uniform phenotype. This heterogeneity in protein expression points towards a tumor origin for these cells. The presence of CD133+, which are early multipotent precursors in the hemangioblastic lineage, strengthens the argument for a tumor origin (tumor "stem cells") rather than mobilization of mature endothelial progenitor cells (EPCs) from the bone marrow. The precise origin of these cells, whether from pre-existing stem cells within the blood vessel lining or those that migrate to blood vessels after transformation, remains unclear. However, the unique properties of the tumor microenvironment likely promote the release of these cells into circulation, contributing to the metastatic spread of hemangiosarcoma.

2.2. Tumor microenvironment

Malignant cells engage in dynamic, two-way interactions with their local microenvironment, playing a pivotal role in regulating their growth and progression. Notably, inflammation and angiogenesis represent recurring hallmarks of HSA. Furthermore, research has demonstrated the expression of functionally active receptors on HSA cells, enabling them to initiate biologically relevant signaling pathways upon binding to specific chemokines. These chemokines include interleukin (IL-8), chemokine (CXC motif) ligand-12 (CXCL12, also known as SDF1α), and even modified sphingosines.

IL-8, a potent pro-inflammatory cytokine, plays a central role in inflammation and angiogenesis within human stromal sarcoma cells. In HSA cells, IL-8 signaling triggers calcium mobilization

and appears to modulate its own gene transcription through a negative feedback mechanism. Similar feedback control may regulate Slug (Snail-2), a transcription factor implicated in stem cell maintenance. Surprisingly, while IL-8 does not directly promote HSA proliferation in vitro, its expression correlates with gene signatures characteristic of reactive tumor microenvironments. This suggests that HSA cells utilize IL-8 production as an adaptive mechanism to shape their surrounding environment (Kim et al., 2014 ; Rodríguez et al., 2015).

The CXCL12/CXCR4 signaling pathway, crucial for both hematopoietic stem cell and cancer cell migration, likely guides HSA cells towards CXCL12-enriched metastatic sites. Gene expression profiling in canine HSA revealed signatures of hematopoietic function and migration linked to CXCR4/CXCL12 expression, bolstering the hypothesis that HSAs arise from hematopoietic precursors. CXCL12/CXCR4 signaling pathway may influence HSA metastasis, but several critical aspects necessitate further investigation. First, the origin of the CXCL12 ligand is unclear. It could be produced by HSA cells themselves (autocrine), by neighboring cells within the tumor microenvironment (paracrine), or even by distant organs (endocrine). Additionally, the role of CXCR4 expressed in inflammatory and stromal cells that contribute to the tumor niche remains undefined. These cells might play a part in the overall signaling mechanisms. Furthermore, it's unknown whether HSA cells actively create CXCL12-enriched environments to attract CXCR4-expressing cancer cells, or conversely, if these CXCR4-expressing cells migrate towards and colonize tissues with pre-existing abundant CXCL12 (Im et al., 2015).

The sphingosine-1-phosphate receptor 1 (S1P1), a bioactive lipid receptor, counteracts pro-apoptotic ceramides and contributes to pathological cell growth. This suggests a potential role for S1P/S1P1 signaling in HSA cell survival (Rodríguez et al., 2015).

Interactions between HSA cells and non-cellular components, such as hyaluronic acids, are believed to play a role in maintaining the inflammatory and angiogenic milieu that supports tumor progression (Kim et al., 2015).

Tumor-associated macrophages, key players in various facets of cancer progression in both human subjects and murine models, are believed to contribute to the immunosuppressive and pro-tumorigenic nature of the tumor microenvironment. In laboratory settings macrophages pivot between alternative polarization, M1 or M2, based on their response to external signals. M1 macrophages exhibit heightened production of proinflammatory interleukins, co-stimulatory molecules, and chemokines, thereby stimulating cytotoxic CD8 T-cell responses

and chemotaxis. Conversely, M2 macrophages generate anti-inflammatory interleukins, angiogenetic factors, and matrix remodeling enzymes. TAMs in humans and mice often exhibit a mixed phenotype, displaying characteristics of both M1 and M2 activation, with a general skewing towards the M2 phenotype. TAMs assume a central role in the tumor microenvironment, being abundantly present in both. Their contribution to tumor invasion, intravasation, survival of tumor cells in circulation, and extravasation is significant. Additionally, TAMs foster tumor progression by promoting genetic instability, remodeling the extracellular matrix, stimulating angiogenesis, and impeding the adaptive immune response. Notably, in metastatic lesions of dogs with HSA, the prevalence of monocytes within the tumor tissue exceeded that in other tumors (De Almeida Monteiro et al., 2018 : Mukai et al., 2020).

A recent study (Gulay et al., 2022) underscored the predominance of CD206-positive infiltrating macrophages in malignant canine mammary tumors, reinforcing the perspective that TAMs in dogs are predominantly M2-skewed and exert a central influence on the tumor microenvironment. Another study examining the prevalence of M2 macrophages within the tumor compared to the surrounding tissue revealed a higher presence of CD204-positive (Tissue-resident and tumor-infiltrating macrophages) and CD206-positive (tumor-infiltrating macrophages) cells in tumor hot spots and the tumor tissue outside of hot spots than in normal surrounding tissues such as spleen, heart, liver, kidneys, and lungs. This suggests a potential increased recruitment and differentiation of monocytes into TAMs within the TME of HSA tumors.

Furthermore, Kerboeuf et al. (2024) observed a significantly higher number of M2-polarized macrophages, identified by CD206 expression, within tumor tissues compared to healthy surrounding tissues. This aligns with similar findings in humans, supporting the concept of TAMs exhibiting immunosuppressive and pro-tumorigenic properties.

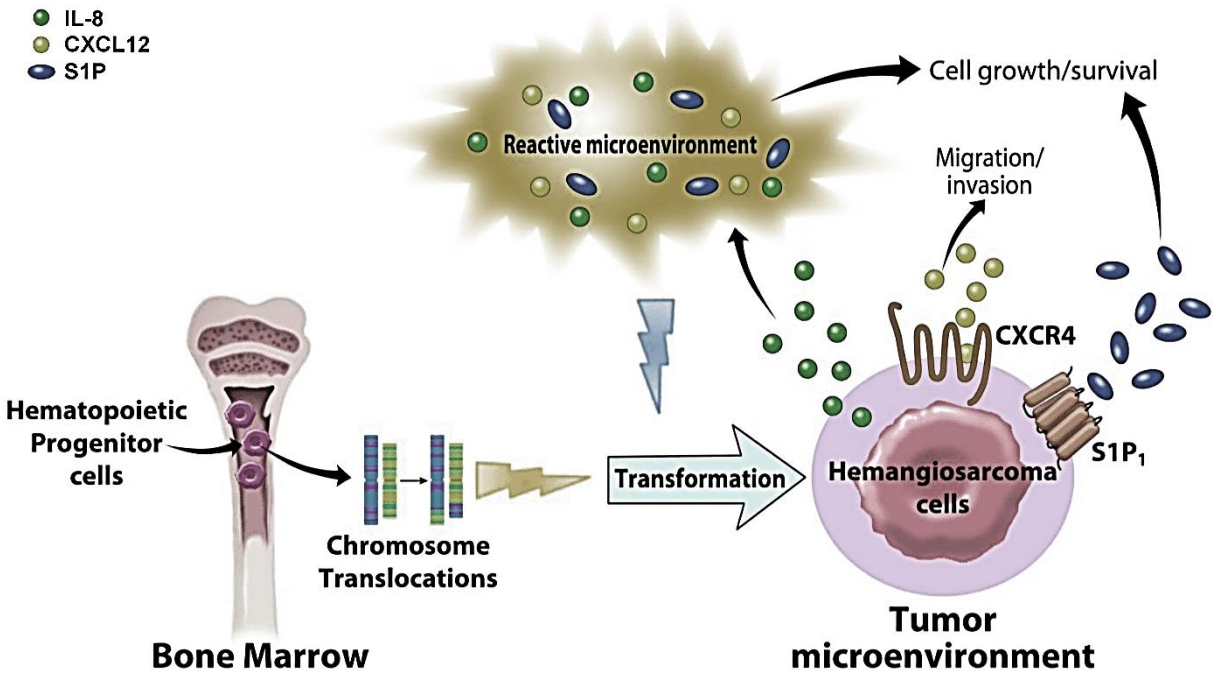

Figure 31. IL-8, produced by HSA cells, is thought to modulate the TME, promoting the growth and survival of tumor cells. CXCR4 and its ligand, CXCL12, found to be abundant in HSA tissue, transduce biological signaling, causing tumor cells to increase their motility to migrate and invade into the other sites for metastasis. Canine HSA cells that consume Sphingosine-1-phosphate (S1P) from the TME induce intracellular signaling through S1P receptor-1 (S1P1), increasing cell growth and survival. It is suggested that these chemokines and modified biolipids are key regulators for HSA behavior (Kim et al., 2015).

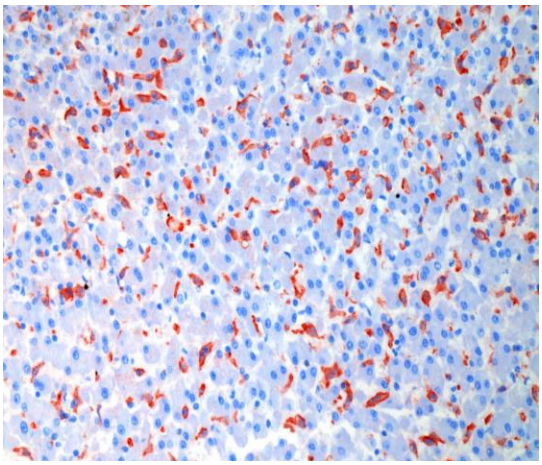

Figure 33. Hemangiosarcoma, surrounding hepatic tissue, CD204 immunolabeling of of Kupffer cells, most tissue resident macrophages in the liver are not immunolabeled for CD206 (Kerboeuf et al., 2023).

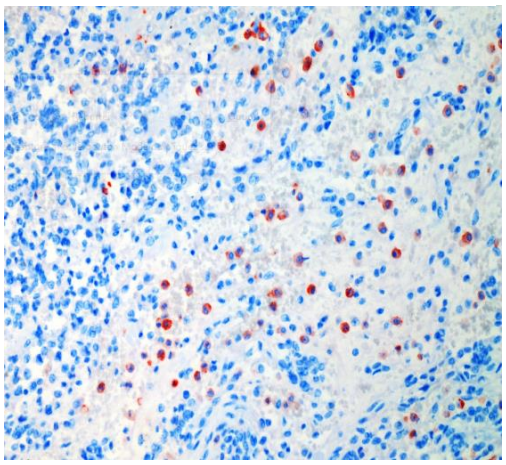

Figure 32. Hemangiosarcoma macrophage hot spot, the majority of tumor-associated macrophages in the liver are immunolabeled for CD206 (Kerboeuf et al., 2023).

2.3. Genetic landscape

A study by Megquier et al. (2019) identified *TP53*, a tumor suppressor gene, as the most frequently mutated gene in canine hemangiosarcoma. These mutations primarily occurred in the DNA-binding domain, suggesting a loss of function for the gene. The study also revealed frequent mutations in the *PI3K* pathway, a crucial signaling pathway involved in cell growth, survival, differentiation, metabolism, and immunity. Nearly half (48.9%) of canine hemangiosarcoma tumors harbored at least one mutation in this pathway, suggesting its potential role in tumor development. Notably, tumors with *PI3K* mutations typically contained only a single mutation within this family. The analysis also identified *RASA1* and *ARPC1A* as potential driver genes based on variant allele fraction and SIFT scores (predicting functional impact of mutations). *RASA1*, a negative regulator of cell proliferation and vascular development, has been linked to human capillary malformation syndromes. *ARPC1A* plays a role in regulating the actin cytoskeleton, which is critical for cell migration and invasiveness in cancers like pancreatic carcinoma. There is less support for a causal role for *ORC1* and ENSCAFG00000017407. Tumors in both dogs and humans were enriched for mutations in protein tyrosine kinases (PTKs), these PTKs act as molecular switches within cells, playing a critical role in regulating signals that control cell growth and division. The high frequency of PTK mutations in canine hemangiosarcoma suggests their potential involvement in tumor development. Interestingly, research also reveals recurrent mutations in another gene family – protein tyrosine phosphatases (PTPs) like *PTEN*. PTPs antagonize PTKs by removing phosphate groups from proteins, effectively turning off the signaling initiated by PTKs. The co-occurrence of mutations in both PTKs and PTPs suggests a potential disruption in their normal regulatory balance. If PTKs are mutated and become overly active, while PTPs are also compromised due to mutations, this could lead to a situation where cellular growth signals remain unchecked, potentially contributing to hemangiosarcoma development. Additionly, phospholipase C proteins play a significant role in cellular signaling cascades by catalyzing the hydrolysis of PIP2, leading to the generation of second messengers DAG and IP3. These messengers transmit signals originating from receptor tyrosine kinases.

In canine HSA, there is an enrichment of phospholipase C genes, notably *PLCG1*. The investigation involved scrutinizing copy number alterations in genes previously implicated in hemangiosarcoma and angiosarcoma. Predominant alterations included copy number gains in VEGFA and KDR, alongside losses in CDKN2A/B. Notably, the *myc* oncogene, which has been documented as amplified in human angiosarcoma and canine hemangiosarcoma, exhibited

infrequent gains in the dataset and lacked substantial evidence of high-level amplification (Megquier et al., 2019 ; Wang et al., 2017 ; Witter, 2015).

ECs detach from existing vessels in a "sprouting phase." These ECs then migrate into surrounding tissues, proliferate, and form new blood vessel tubes during the "branching phase". Finally, in the "pruning phase," excess immature vessels are meticulously eliminated through a process called apoptosis, which is normally counteracted by anti-apoptotic factors like bcl-2 (encoded by *BAK1*) and surviving. This delicate balance between vessel formation and removal ensures proper tissue repair and regeneration (Bishop et al., 1999 ; Duval et al., 2003).

HSA shows high expression of bcl-2 and survivin proteins similar to actively growing blood vessels during angiogenesis, his suggests that malignant endothelial cells (ECs) in HSA evade cell death signals through a mechanism resembling active angiogenesis (Oungsakul, 2020)

Genetic analysis of canine hemangiosarcoma revealed significant copy number gains in genes belonging to the angiopoietin family. These genes play a critical role in regulating angiogenesis, endothelial cell survival, proliferation, and migration. ANGPT1 and ANGPT4 genes that encode ligands for the TIE2 receptor, expressed on vascular endothelial cells. Binding of ANGPT1 and ANGPT4 to TIE2 promotes endothelial cell survival, proliferation, and blood vessel stability, essentially acting as pro-angiogenic factors, ANGPT2 gene product typically functions as an antagonist to the pro-angiogenic factors, promoting endothelial cell death and blood vessel regression. Interestingly, the study found recurrent genomic gains in chromosomes harboring both ANGPT1 and ANGPT4 genes, suggesting a potential upregulation of pro-angiogenic signaling in hemangiosarcoma. Notably, ANGPT4 displayed a particularly high incidence of gain in Australian Shepherd Dogs (70% compared to a 31% average in other breeds). In contrast, the gene encoding ANGPT2, the anti-angiogenic ligand, was located on a chromosome with the highest frequency of global deletion across the entire hemangiosarcoma cohort. However, the incidence of specific copy number loss within the ANGPT2 gene itself was relatively low (20% of all cases). This pattern of genetic alterations suggests a potential imbalance favoring pro-angiogenic signaling pathways in canine hemangiosarcoma, potentially contributing to the excessive blood vessel growth characteristic of this disease. The SKI oncogene emerged as a strong candidate for a gene involved in the 21% of hemangiosarcoma cases exhibiting copy number gain (Thomas et al., 2014 ; Cascone and Heymach, 2012).

SKI acts as a negative regulator of the TGFβ signaling pathway, which plays a crucial role in cell growth and differentiation (Bonnon et Atanasoski, 2012). Upregulation of *SKI* has been

observed in various human cancers, promoting tumor growth, stimulating angiogenesis, and often correlating with a poorer prognosis. Adding to the potential significance of *SKI*, research suggests a similar link between *SKI* and human pediatric hemangiomas, with the highest *SKI* expression observed in the most actively proliferating tumors. However, the specific mechanisms by which *SKI* dysregulation occurs in malignant cells remain unclear and require further investigation (Thomas, 2014).

Recent study by Gorden et al. (2014) supports previous research on cell lines, suggesting that angiogenesis and inflammation play a key role in hemangiosarcoma development, showed that HSAs could be stratified according to the expression of pro-inflammatory genes, endothelial cell-matrix interaction genes , or pro-adipogenic and connective tissue-forming genes.

The three distinct molecular subtypes based on gene expression analysis:

- **Angiogenesis Subtype:** it exhibits a strong expression of genes associated with functions of blood vessel development, angiogenesis, vasculogenesis, endothelial cell development, and migration

- **Inflammation Subtype:** shows increased expression of genes involved in inflammatory processes, immune cell differentiation, homeostasis and development, and migration.

- **Adipogenesis Subtype:** displays gene expression patterns related to lipid, cholesterol transport, fatty acid, cholesterol, and steroid metabolism.

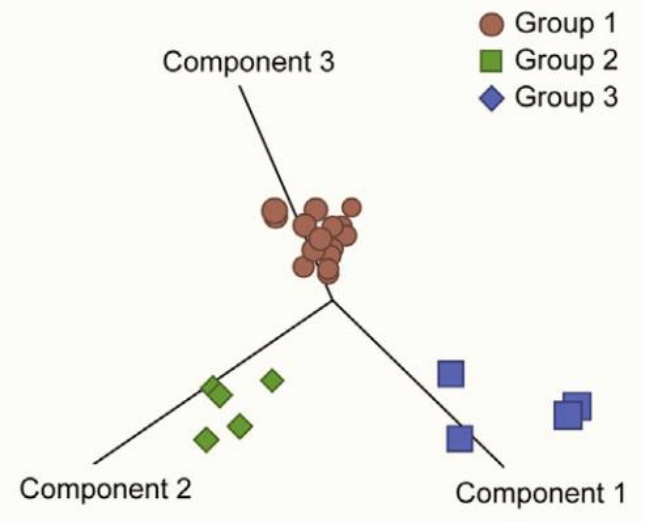

Figure 34. Genome-wide expression analysis identified three molecular subtypes in hemangiosarcoma, genes with variance >0.5 across 24 samples were used to generate principal component analyses and hierarchical clustering, samples were assigned to one of three groups by unsupervised clustering to identify genes with significantly different expression between groups (Gorden et al., 2014).

Gene ID	Indels	SNVs	Total mutations	Covd Bps	Muts pMbp	FDR
TP53	3	25	28	72,582	386	0
PIK3CA	0	14	14	156,415	90	0
PIK3R1	0	4	4	124,857	32	5.9×10^{4}
ORC1	0	4	4	133,219	30	8.2×10^{4}
RASA1	0	4	4	136,539	29	1.6×10^{4}
ARPC1A	0	3	3	72,351	41	5.1×10^{4}
ENSCAFG00000017407	0	2	2	22,074	91	5.9×10^{4}

Table 10. Significantly mutated genes in golden retriever hemangiosarcoma tumors ; Significantly mutated genes have an FDR < 0.1.58 (Megquier et al., 2019).
Abbreviations: Covd Bps, number of basepairs with adequate coverage in gene; SNV, single nucleotide variants; Muts pMbp, mutations per megabase; Indels, insertion-deletion.

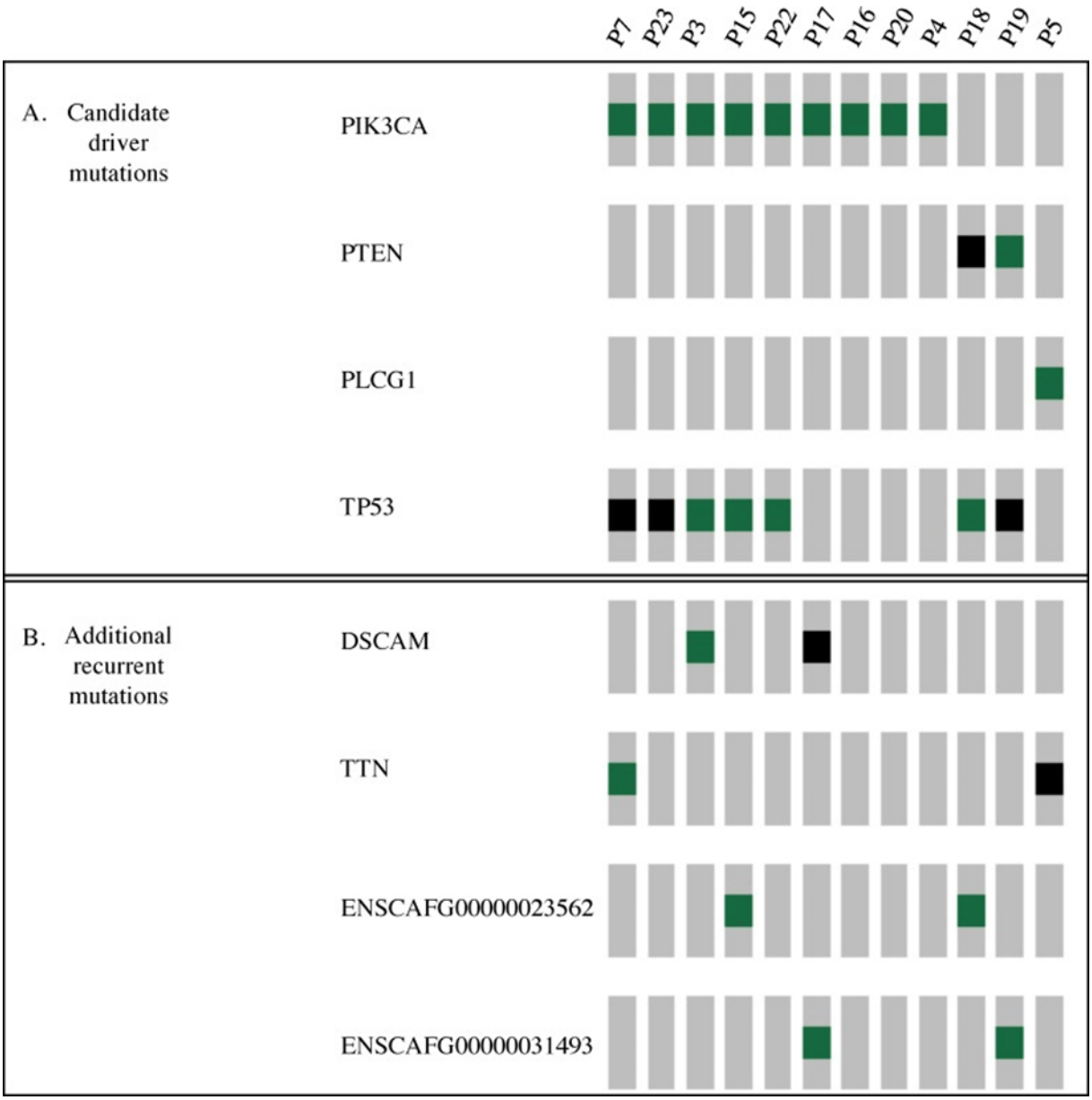

Figure 35. Candidate driver mutations and recurrent mutations. Each row represents a mutated gene and each column represents an individual tumor. Shown in green blocks are non-synonymous, non truncating variants which could represent gain-of-function mutations. Shown in black are predicted inactivating mutations, including truncating mutations, essential splice-site variants, and nonsense mutations (Wang et al., 2017).

2.4.Paraneoplastic syndromes

2.4.1. Hypercoagulopathy

Canine and human cancer patients frequently experience blood clotting complications, such as thrombosis and DIC, which significantly worsen their prognosis. In the context of hypercoagulability associated with malignant tumors, tissue factor (TF) assumes a pivotal role,serving as the primary initiator of the extrinsic coagulation cascade. Aberrant expression of TF is evident in numerous human cancers. Notably, TF is discernible on microparticles (MPs) discharged from diverse cell types, including tumor cells, monocytes, endothelial cells, and potentially platelets. These minuscule vesicles (0.1–1.0 μm), originating during cellular activation or damage, encapsulate various proteins, phospholipids, and nucleic acids (mRNA and microRNA) from their parent cells, establishing their significance as pivotal mediators in pathological processes. Irrespective of their origin, circulating TF-bearing MPs (TF-MPs) possess the ability to instigate blood coagulation by forming complexes with factor VII (FVII) or activated FVII (FVIIa) in the plasma. Investigations in canines have documented TF expression and TF procoagulant activity in cell lines associated with mammary gland tumor, pancreatic carcinoma, prostatic carcinoma, bronchoalveolar carcinoma, osteosarcoma, fibrosarcoma, and hemangiosarcoma (Heishima et al., 2015 ; Kobayashi et al., 2019 ; Maruyama et al., 2005).

Studies conducted by Witter et al. (2017) have delineated that hemangiosarcoma cell lines derived from primary tumors in canines consistently express TF mRNA and exhibit TF antigen on their cell surfaces. In comparison to non-neoplastic canine aortic endothelial cells (CnAoEC), these hemangiosarcoma cell lines induce significantly heightened thrombin generation (a pivotal clotting factor) in canine plasma in vitro, as assessed by the calibrated automated thrombography (CAT) assay. This escalated thrombin generation is instigated by the extrinsic coagulation pathway and is contingent upon factors such as the presence of FVII, cell number (influencing available surface TF), and the expression of phosphatidylserine on the cell membrane. The research provide substantive evidence supporting the notion that the aberrant expression of procoagulant tissue factor (TF) on hemangiosarcoma tumor cells plays a contributory role in this paraneoplastic syndrome.

2.4.2. Non-islet cell tumor hypoglycemia

Leiomyosarcoma and leiomyoma of the gastrointestinal tract and liver are the most frequent mesenchymal tumors, but also has been described in dogs with splenic hemangiosarcoma (Zini et al., 2007). Different mechanisms are hypothesized to explain NICTH, this PTS is

predominantly associated with tumor production of the prohormones insulin-like growth factor I and insulin-like growth factor II, mechanisms of IGF-II on lowering blood glucose are mainly mediated by its insulin-like activity due to a 50% similarity to insulin (biologic activity very similar to that of insulin without concurrent elevations of serum insulin levels), but also in part by its inhibitory effect on glucagon (Phillips et Robertson, 1993 ; Maki, 2010).

2.4.3. Microangiopathic hemolytic anemia

MAHA can develop in conditions where red blood cells are subjected to shear stress due to passage through abnormal or constricted blood vessels, or exposed to turbulent blood flow. Schistocytes, erythrocytes with fragmented, pointed projections, are a characteristic finding in MAHA. This mechanical fragmentation of red blood cells is particularly observed in animals, especially dogs, with disseminated intravascular coagulation as erythrocytes become entangled and disrupted within the fibrin meshwork of microthrombi. Fragmentation anemia, a consequence of MAHA, is a frequent complication in canine hemangiosarcoma (Rebar et al., 1981).

Discussion

Canine hemangiosarcoma remains a significant challenge in veterinary oncology due to its aggressive nature and limited treatment efficacy. This comprehensive literature review has shed light on various facets of HSA pathobiology, providing valuable insights into diagnosis, potential therapeutic targets, and areas demanding further investigation.

Key Findings

- Establishing a definitive diagnosis of HSA relies on a combination of techniques, including cytology, histopathology, and hematology. However, each method has limitations. For instance, cytology may yield inconclusive results due to sample quality or bleeding complications Histopathology remains the gold standard for diagnosis, but its invasive nature can be a drawback. Emerging biomarkers, such as circulating tumor cells and cell-free DNA, offer promise for minimally invasive and potentially earlier diagnosis of HSA.

- The intricate sequence of events leading to HSA development involves a complex interplay between genetic predispositions, dysregulation of cellular pathways, and interactions with the tumor microenvironment. Specific genetic mutations, such as those affecting *p53* and *PTEN* tumor suppressor genes, have been associated with HSA development. Additionally, dysregulation of signaling pathways, including the PI3K and *Ras* pathways, play a critical role in HSA pathogenesis by promoting uncontrolled proliferation and survival of malignant endothelial cells. Furthermore, the tumor microenvironment, composed of surrounding stromal cells, immune cells, and blood vessels, actively contributes to HSA progression by providing growth factors, promoting angiogenesis, and suppressing anti-tumor immune responses.

Future Directions

- Continued research is necessary to validate and implement novel diagnostic biomarkers for early detection of HSA, ultimately improving patient prognosis. This includes refining existing methods for circulating tumor cells and cell-free DNA detection and exploring the potential of additional biomarkers.

- Elucidating the specific roles of genetic mutations in HSA development and their interactions with signaling pathways holds immense significance. Functional studies

investigating the downstream effects of these mutations and their impact on cellular processes can pave the way for targeted therapies.

- Investigating the tumor microenvironment of HSA and its influence on tumor progression and metastasis is critical for developing therapeutic strategies that can target not only the tumor cells themselves but also the supportive microenvironment. This might involve manipulating the composition of the tumor microenvironment to enhance the efficacy of existing therapies or developing novel drugs that target specific components within the microenvironment.

- Translation of research findings into improved treatment options for HSA patients requires further investigation and clinical trials. This could encompass targeted therapies that specifically inhibit dysregulated signaling pathways or immunotherapies that harness the body's immune system to combat the tumor.

By addressing these areas of future research, scientists and veterinarians can improve diagnostic accuracy, identify novel therapeutic targets, and ultimately enhance the prognosis for dogs diagnosed with hemangiosarcoma.

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